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FACTORS AFFECTING GROWTH FROM THE FOLIAR MERISTEMS OF BRYOPHYLLUM CALYCINUM

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(WITH FOUR FIGURES)

Introduction

At the base of each marginal notch of the leaf of *Bryophyllum calycinum* is a group of cells which, under favorable conditions, may produce an entire new plant. A study correlating the activity of these cells with the functions of those adjacent, and with the several plant organs, demonstrated, according to LOEB, certain mass relations. These relations he stated were the only precise logical explanation for regeneration in plants and animals.

Studies by REED (31), OSSENBECK (26), KAKESITA (19), and others have presented evidence contrary to the findings of LOEB. It seemed advisable to attempt a reinterpretation of the available data, to test the validity of current hypotheses, and to study certain of the chemical and enzymatic changes which accompany growth from the foliar meristems.

Materials

The plant used, *Bryophyllum calycinum* Salisb. (*Br. germinans* Blanco; *Br. pinnatum* Kurz.), is the same species as that employed by GOEBEL, DE VRIES, REED, OSSENBECK, and others. Five collections from the following sources were used: (1) the conservatory at Golden Gate Park, California; (2) a private garden in Santa Barbara, California; (3) the University of California, Berkeley; (4) a

collection at the University of Wisconsin; and (5) materials of the same lot as those used by REED (31), obtained from Professor H. H. BARTLETT of the University of Michigan. Collection 5, bearing the label "University of Michigan Botanic Gardens 1761," was selected as standard for these studies.

Varietal differences

There are indications of varietal differences of a degree sufficiently great to account for the lack of uniformity in observations made by investigators in the past. Plants from collections 4 and 5, when placed in a darkroom ventilated with greenhouse air, under conditions which were abnormal only in the absence of light, showed in 10 days a random distribution of proliferation from notches of all leaves except those of the upper three nodes. No proliferation was observed on any plant of collection 1 or 3 under identical conditions. These observations may explain why OSSENBECK (26) was unable to verify positive results reported by REED regarding the activating effect of darkness. Root systems of all plants used in the present study were carefully examined, and all were found to be healthy and functioning.

Another constant varietal or strain difference was manifest in the rooting potentialities of petioles from plants in lots 1 and 4 on the one hand, and of those in lot 5 on the other hand. At no time during many trials, which involved the use of a wide variety of media, were any roots produced from the petioles of plants composing lots 1 and 4. In every experiment with leaves from plants of lot 5, roots were obtained from some of the petioles. It was apparent that leaves from different clones of the same species differ in the distribution of toti-potential cells.

Morphology

The single recent publication regarding the anatomy of the leaf notches is a short and rather inconclusive statement by BEALS (3). Camera lucida drawings of sections of notches are presented, with the conclusion that the roots and shoots "arise from the division of small phloem cells near the vegetative points or notches of the leaves." Morphological studies have shown the leaf to be bifacial and to possess no palisade. Stomata are present on both surfaces and are surrounded by subsidiary cells.

There is a probable chemical difference in cells which respectively compose the abaxial and adaxial halves of a leaf. Young leaves stained with Flemming's triple stain after fixation with formalin-acetic-alcohol appear to be divided through the center by a region of vascular tissue. On each side are small parenchymatous cells which although they do not noticeably differ morphologically from one another, behave differently toward stains. Such differences in staining have also been noticed on preparations made with simple stains, such as gentian violet.

A definite polarity of dorsiventral nature is observable in the proliferating notch. Roots invariably arise from the abaxial side of the leaf regardless of its position, and leafy shoots always arise from its adaxial surface.

Studies were made of the anatomy of the developing leaf to determine whether or not the meristems which give rise to new roots and shoots are formed normally in the development of the leaf, or only following unusual stimulation of the plant. No effort was made to trace the condition further than to situations which exist in leaves one-eighth of an inch in length. At this stage of development the leaf will not proliferate, even under the most favorable experimental conditions. In leaves this size marginal, subepidermal meristems composed of a number of nearly isodiametric cells are noticeable. No observation has been made in the present study which would suggest that cells which compose the foliar meristems are at any time more than embryonic. Thus it does not appear that proliferation in *Bryophyllum* involves the changing of differentiated cells to the embryonic condition before other changes occur. It does appear, however, that the notch meristems are differentiated extremely early in the normal development of the leaf, much as axillary buds are formed in the normal development of the stem. During the time in which the surrounding cells differentiate into the tissues of the mature leaf, the cells which are later to form the new plants remain in an embryonic condition, although their numbers increase, as may readily be observed. Well developed vascular regions are found adjacent to the meristems. Such a coincidence may have suggested to BEALS that the meristems have their origin in these vascular regions, but no evidence has yet been presented to demonstrate beyond doubt

that these veins and meristems may not have wholly separate origins.

Current hypotheses of regeneration

Proliferation from leaf notches of *Bryophyllum* has been explained by hypotheses of four types, involving: (1) formative stuffs; (2) inhibitive substances; (3) altered nutrient relations; and (4) anaerobic respiration.

According to the hypothesis of formative stuffs, applied to this problem by LOEB, specific hormones are synthesized in the leaves of *Bryophyllum* plants, irrespective of attachment of the leaf to the plant. When the leaf is a functional part of the plant, these hormones are removed by the growing points of the stem and the roots; hence an accumulation of sufficient quantities of hormones to activate the notch meristems cannot occur. If the leaf is separated from the plant, however, the hormones accumulate and stimulate the production of new plants.

To establish the hypothesis of formative stuffs, it must be shown that proliferation occurs only in the absence of root and shoot growing points, and that it never occurs in their presence. Experimental data are at hand to demonstrate the inadequacy of such a hypothesis. Numerous cases of proliferation which have occurred in the presence of functioning root and shoot apices will be cited later. The present study has also verified the experimental results of REED (31), that a leaf may be removed from the influence of both root and shoot apices for a time without stimulating the marginal notches to activity. Moreover, LOEB'S theory fails to explain the following:

1. Attached leaves of unmutilated plants have been observed to proliferate by REED (31), RENICH (32), SMITH (35), BRAUN (7), FYSON (12), and even by LOEB himself (24). Such cases of so-called regeneration have been observed by the writer in material used at California and Wisconsin, and in the field, under natural conditions, in Honolulu (fig. 1).

2. Numerous experiments[1] have demonstrated that the shoot

[1] All experiments were conducted in a greenhouse in which there were no artificial gas outlets, and in which plants extremely sensitive to ethylene or coal gas showed not the slightest injury.

apex which, according to LOEB, removes the formative stuffs from the leaves and thus keeps them dormant, may be removed without stimulating proliferation. Growth from the axillary buds (axils of leaf as a whole) replace the apex.

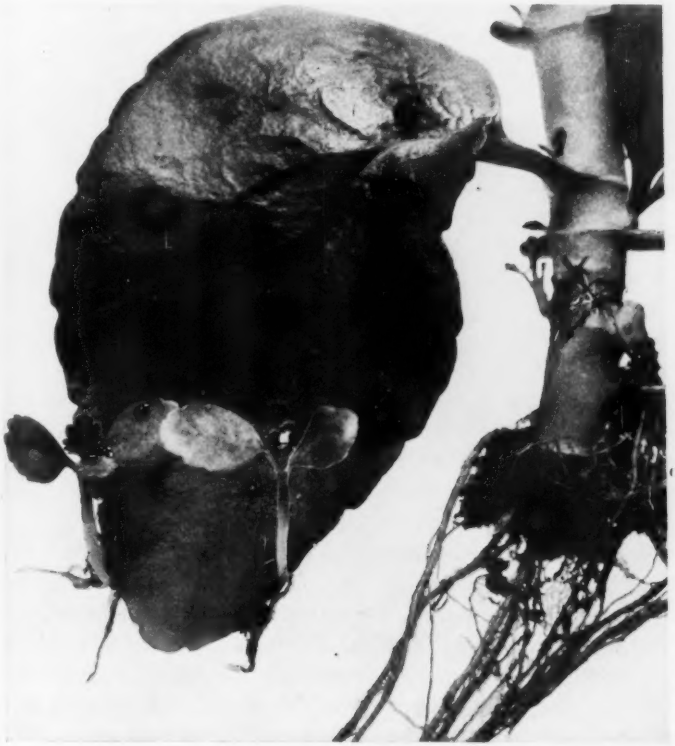

FIG. 1.—Proliferation of attached leaves of young vigorously growing plants; growth occurs from notches in contact with moist soil; no special treatment used (photography by courtesy of J. P. BENNETT).

3. Root growth has been inhibited for long periods by encasing the roots in plaster of Paris casts. Shoot growth was limited simultaneously without causing the growth of foliar meristems.

4. While leaves were attached to plants, growth was induced at the notches by the following means: (1) immersion of leaves in water

(WAKKER **38** and REED); (2) making a single incision through the midvein of the leaf (GOEBEL **13**); (3) darkness (REED **31**)*; (4) crown-gall inoculations (SMITH **35**)*; (5) building up of moist sand to the lower surfaces of attached leaves (REED)*; (6) warm bath (KAKESITA **19**); (7) hydrogen atmosphere for 48 hours (KAKESITA)· (8) chemical injections (KAKESITA); (9) etherization (GOEBEL **13**)*; (10) prolonged saturation of soil (Experiment I)*; (11) extended drought (Experiment II)*; (12) ringing plants by removal of fleshy cortex and phloem (GOEBEL **13**)*; (13) wrapping stems tightly with thread (Experiment III)*; (14) injections of tissue juice (Experiment IV)*.

EXPERIMENT I.—An observation of FYSON (**12**) that a number of plants growing in open fields in India showed proliferation from the leaf notches after a long rainy period suggested that the same results might be brought about experimentally by excessive moisture about the roots. Accordingly vigorous potted plants were placed in glass vessels of boiled water to a depth sufficient to cover the soil by 2 inches. Heavily paraffined wrapping paper was fastened securely over the top of the vessel and around the base of the stem, which protruded centrally from the dish. Within one week roots were growing from the notches of the leaves of all plants used. Later roots were also produced on the stems above the water level.

EXPERIMENT II.—Drought under natural conditions has been noted by many to have an activating effect upon buds in general. This fact, together with evidence that ether exerted a stimulating effect upon the leaf meristems of *Bryophyllum*, seemed to indicate that insufficient water might have a noticeable effect on the activity of the notches. Several plants were therefore set aside to dry for 5 weeks. Control plants of the same age and variety were placed in a similar situation but received water daily. Within the experimental period almost every leaf of the dry plants except those of the top two nodes showed root development. Many repetitions of this experiment gave similar results.

EXPERIMENT III.—Around each of six small plants 4 months old 8–10 yards of white silk thread was wound tightly at an internode

* These methods of stimulating proliferation have been used successfully in the present study.

some distance above the soil. During the following 5 months, increase in girth of the stems left a considerable constriction at the point about which the thread was wrapped. Above this constriction the stem was of more than normal diameter. Shoot growth took

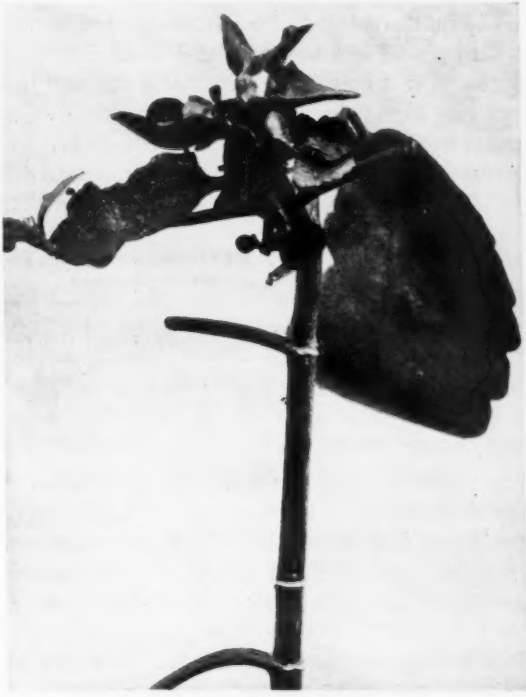

FIG. 2.—Proliferation of apical leaves of vigorously growing plant induced by tightly wrapping stem with thread; some leaves removed at time of photographing.

place from the leaves above the constriction; on leaves of control plants no such growth occurred (fig. 2).

EXPERIMENT IV.—While there is an extensive literature regarding the effects of accumulations of hormones upon the activity of foliar meristems of *Bryophyllum*, it is pertinent to note that an experimental study to demonstrate the actual presence of such hormones has never been made. For such a study, tissue adjacent to

and including the notches was removed from a total of 150 notch areas. The leaves for these notch areas were separated from the plants for a period long enough to show slight proliferation. Sister leaves (leaves opposite the ones removed) were marked with tags, and a similar number of dormant notches with adjacent tissue were removed. The dormant and active notches were separately frozen. Each was then placed in a mortar with a small amount of quartz sand, and ground to a pulp. Ten cc. of water was used to wash the pulp of each into hard glass beakers. Hydrogen-ion determinations of each extract were made, and the beakers then set in a refrigerated room for 72 hours at a temperature of 10° C. At the end of this time

TABLE I

EFFECT ON DORMANT LEAF MERISTEMS OF INJECTION OF TISSUE JUICE EXPRESSED FROM ACTIVE AND DORMANT NOTCHES

NOTCH TYPE USED AS INOCULUM	pH. OF INOCULUM	DATE OF INOCULATION	DATE OF OBSERVATION	RESULTS
Active	6.15	2/6/30	3/10/30	Small shoot growth from number of notches
Dormant	6.10	2/6/30	3/10/30	

the water and residue were stirred together and the mixture centrifuged. Small amounts of the supernatant liquid were injected into tissue adjacent to dormant notches of leaves of vigorous plants. The injection was made by means of a hypodermic syringe, which was of glass except the needle. Table I shows the results of the experiment.

There is no evidence at hand to demonstrate that the water-soluble fraction of dormant and active notches differs in any respect with regard to the presence of activating hormones. The incompleteness of this line of experimental evidence is thoroughly recognized, however, but it may be said that the LOEB hypothesis of formative stuffs is entirely without verification in any experiment of the present study. A theory of inhibiting substances, such as LOEB later advocated (21), is also untenable for similar reasons. In all events the present theory of hormonal control of proliferation is unsatisfying, since to be of intrinsic value it must deal with material changes of demonstrable types.

On the basis of mass relationship studies, LOEB (**24**) stated that photosynthetic materials of the usual nature instead of hormones are probably the cause of proliferation:

> To complete the proof that we are dealing here with the action of the quantity of material produced by assimilation it remains to be shown that the results described occur only in the presence of light, while in the dark the production of roots and shoots in an isolated leaf of *Bryophyllum* is negligible. . . . . This explains in part why in the normal plant no regeneration of roots and shoots occurs in the leaf. In the normal plant all material is used either for growth of the leaf or growth of the stem apex, roots, and stem itself, as will be seen later.

The results of the writer's experiments do not lend themselves to this interpretation. First, proliferation from foliar notches not only does occur in the dark, but a higher percentage occurs in the dark during a given period than occurs in constant illumination (sunlight supplemented by two 500-watt Mazda lamps 3 feet above, constantly burning), and, second, leaves or stem pieces which have been kept in constant darkness for 47 days will produce roots and shoots without subsequent exposure to light. Thus in experimental plants the absence of photosynthetic activity for 7 weeks, during which time two internodes elongated in each plant, there still remained sufficient materials of proper type to permit proliferation from the foliar notches.

KAKESITA (**19**) indicated that intermediate products of anaerobic respiration were the cause of proliferation of leaf meristems. Briefly stated, he demonstrated that notches may be activated by the Molisch warm-bath treatment, which treatment, according to BORESCH, induces intramolecular respiration. An atmosphere of hydrogen for 48–72 hours was found sufficient to cause activation by an accumulation of chemical materials of a type formed during alcoholic fermentation by yeasts. These results appeared to be verified by chemical injections of intermediate and end products of alcoholic fermentation.

A critical review of KAKESITA's data and methods has been made. First, owing perhaps to the preliminary nature of the paper, KAKESITA's methods were not given in detail. The method of applying the Molisch warm-bath treatment may have been one of two ways. The entire plant may have been submerged in such a manner

that the soil was water-logged, or it may have been inverted over the water so that the entire plant above the soil line was treated while the soil still contained no more than the original amount of water. Such a variation of method may be of crucial importance in the results obtained. Presumably, in the absence of a statement to the contrary, the soil was saturated. The method of "carefully considering a supply of fresh air" for the plants subsequent to treatment is not elucidated, yet this factor is of prime importance for a correct interpretation of the effects due to the period of treatment. The same criticism may be made of the studies to determine the effect of an atmosphere of hydrogen on proliferation. These plants were "covered with bell jars and were given a full supply of fresh air." The conditions subsequent to treatment are too vaguely presented.

If anaerobic respiration does cause proliferation, a relatively inactive gas, such as nitrogen, should produce a positive effect when used as an atmosphere to exclude oxygen. To avoid confusion in distinguishing results which might be due to the absence of oxygen from those which are due to an accumulation of carbon-dioxide, a flow of gas sufficient to keep the concentration of carbon-dioxide at a minimum must be used. In my work special apparatus was designed to make possible such flow of gases, and to eliminate difficulties due to insufficient moisture. Fig. 3 is a diagram of the ventilated moist chamber unit which was used in the present series of experiments.

In November, 1929, an attempt was made to study the effect upon the leaf notches of plants of atmospheres of air (1) minus carbon-dioxide; (2) minus carbon-dioxide and oxygen (nitrogen was used); and (3) unaltered except that it was washed by bubbling through distilled water in constant light, constant darkness, and intermittent illumination (12 hours per day of each). The apparatus consisted of nine moist chambers of the type diagrammed in fig. 3. These were arranged in series of three chambers each, and were separated from one another by gas washing bottles of 500 cc. capacity. Series *A* consisted of three chambers in which the plants were treated for 124 hours with air washed through a 60 per cent solution of potassium hydroxide to remove the carbon-dioxide. Series *B* consisted

of three similar chambers in which the plants were treated for the same period with air washed through bottles of distilled water. Plants of series *C* were treated with nitrogen washed through alkaline pyrogallate solution (pyrogallic acid 22 per cent, KOH 60 per cent). In each series the three conditions of illumination were maintained as illustrated in fig. 4. Constant darkness was obtained by painting the bell jars with three coats of dull black Japalac. Constant light was obtained by using a 500-watt Mazda lamp suspended 2 feet above the center control chamber. Intermittent light was provided by means of fiber board hoods, shown in fig. 4. These were painted black on the inside and white on the outside, and were of such a height that they permitted a flow of gas through the inlet tubulatures of the moist chambers without interference. As shown in fig. 4, the bases were buried in builder's sand to a depth of 2 inches. Beneath the hoods the outlet tubulatures passed through the sand in such manner that they were not disturbed by removal

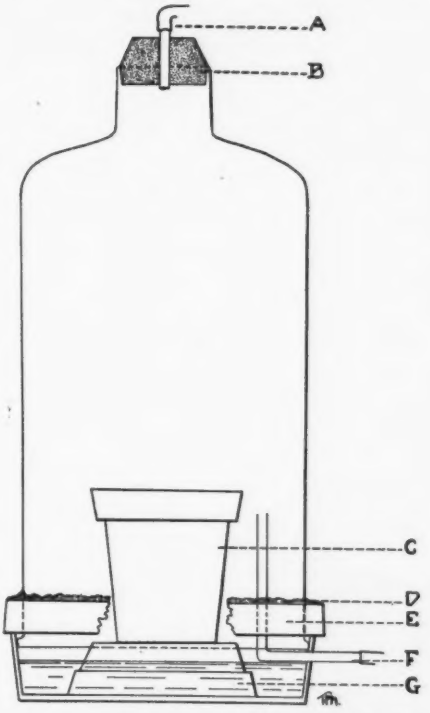

FIG. 3.—Diagram of ventilated moist chamber unit: *A*, glass tube inlet with rubber connection to gas source; *B*, rubber stopper securely shellacked in place; *E*, large clay saucer saturated with paraffin and sealed tightly to adjoining bell jar by seal *D* (composed of plasticine painted over with paraffin); *C*, potted plant resting on inverted clay saucer *G* to prevent saturation of soil by water in *E*; *F*, outlet tube connected either to wash bottle or to water-jet vacuum pump.

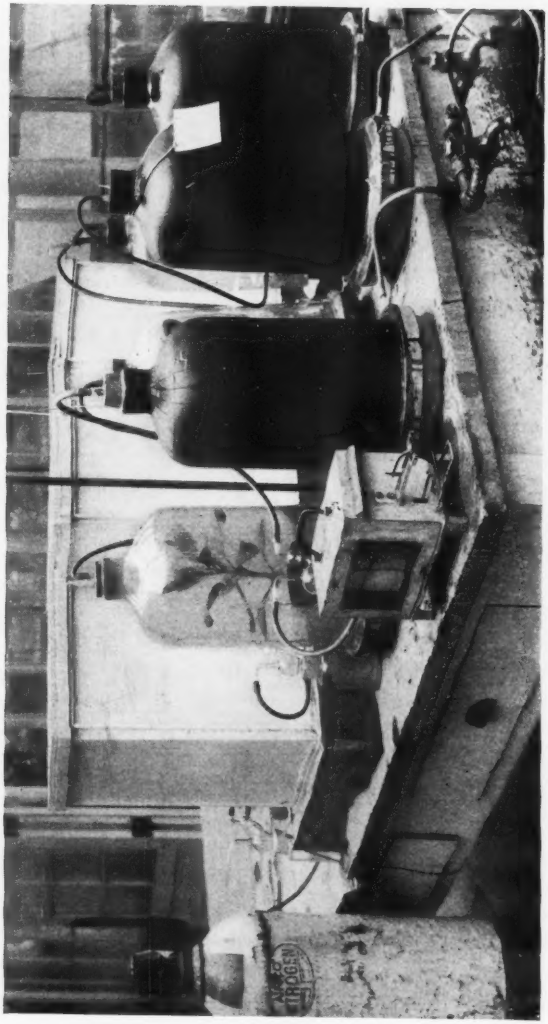

FIG. 4.—Illustration of apparatus used

of the hoods. Temperatures during the 124-hour gas treatment were recorded automatically, and varied from 67° to 83° F. After the 124-hour period the nitrogen was discontinued, the pyrogallate was replaced with clean wash bottles of distilled water, and the series was attached to a water jet vacuum pump (fig. 4). In a similar manner the KOH solution of series *A* was displaced with distilled water. During the remaining days of the experiment the temperatures reached the abnormally high point of 90° F. on the eleventh day, and a low point of 54° F. on the seventeenth day. The average temperature was ±70° F. During the entire experimental period gas was moving through the chambers at the rate of 6–7 liters an hour: thus a complete change of atmosphere presumably occurred every 2 hours.

In contrast with the gas experiments by KAKESITA, the following differences should be borne in mind: (1) nitrogen instead of hydrogen was used; (2) the period of treatment in artificial atmospheres was extended to 124 hours in contrast to the 72-hour period of KAKESITA; (3) a method was used to eliminate high concentrations of carbon-dioxide by constantly changing the atmospheres once every 2 hours; (4) three conditions of light were observed; (5) nine separate combinations of gas and light factors were made possible simultaneously; (6) the methods made possible a separate determination of the effect of absence of oxygen and absence of carbon-dioxide on proliferation; (7) the limiting effect of absence of moisture was removed.

A repetition of this experiment, in which the period of exposure to nitrogen was reduced to 72 hours, gave results similar to those of the longer exposure. In both instances the condition which should favor intramolecular respiration, namely, an atmosphere of nitrogen, did not noticeably increase the occurrence of proliferation from the leaf notches of the experimental plants. In all conditions of light and in all atmospheres, some proliferation occurred. Moisture seemed to exert a controlling influence.

The Molisch warm-bath treatment has been reported by KAKESITA to be a successful means of stimulating growth from foliar notches of *Bryophyllum*. OSSENBECK previously reported negative results from similar attempts. In the present study, unmutilated vigorous plants suspended over a water bath for 10 hours at a tem-

perature varying from 30° to 35° C., in such a way that the soil was not wet, showed no activating effect of the warm bath. It seems that the Molisch bath in itself is not the cause of proliferation in *Bryophyllum*, but that conditions following the treatment may cause the activation, especially very moist conditions.

Several means of causing proliferation do not appear to support the KAKESITA hypothesis: (1) activation of leaf notches by cooling roots under conditions that cause no permanent injury to the roots (OSSENBECK **26**); (2) darkness (REED **31**)*; (3) cutting the midvein of a leaf in such a way that under similar conditions wounding of large areas of leaf tissue between the major veins exerted no influence (GOEBEL)*; (4) drought. All these environmental conditions may have other effects in common. They would conceivably have an appreciable effect upon carbohydrate synthesis, and it seems that many of the observed effects of changed environmental conditions may be more readily explained on the basis of their effect upon photosynthesis than as causing anaerobic respiration.

Since experiments performed by the writer and by others fail to support two of the fundamental bases of KAKESITA'S hypothesis, it is felt that this hypothesis cannot be strongly supported as a general explanation of proliferation of foliar meristems of *Bryophyllum*.

Enzyme studies

Of the standard methods available for activating dormant buds, tubers, and seeds, the following have given positive results in stimulating dormant notches of *Bryophyllum:* treatment with ether, mechanical stimulation by needle pricks, ringing of the stem with a knife, tightly binding the stem with thread, and drought. Other methods which have been ineffective in the present study are the Molisch warm-bath treatment, injection of ethyl alcohol, organic and inorganic acids, and injection of the POPOFF (**30**) "cell stimulants." The consensus of opinion seems to be that the activation of dormant structures by these means is due to their effect on the enzymes, either directly or indirectly (**2, 6, 9, 11, 17, 18, 27, 37**).

A complete series of experiments to throw light on the enzyme relations of activated and dormant notches and tissue adjacent to

* These experiments were successfully repeated in the present study.

these in the leaves of *Bryophyllum* were planned. At the present time only a preliminary statement can be made regarding the carbohydrases and catalase.

LOEW (**25**) described the enzyme catalase. Two types of catalase were recognized, alpha and beta. Alpha catalase is probably a compound of the soluble catalase beta with a nucleo-protein, while beta is an albuminose and can be liberated from the combination by the action of very dilute alkali. Studies to determine the relative activities of catalase from plant tissues must be planned with great care to overcome many sources of error (**1, 15, 16, 29**).

The apparatus used was a modification of the APPLEMAN apparatus (**1**). This modification was as follows: (1) the use of glass stopcocks throughout; (2) coloring the water used in the manometer by the addition of a trace of fast green in alcohol; (3) the insertion of a U-tube of soda lime between the reaction chamber and the manometer to remove any carbon-dioxide; and (4) the use of a mechanical shaker. This shaker was operated off the agitator in the constant temperature bath in which the reaction chamber, a 500 cc. round bottom, Pyrex flask, was immersed.

The hydrogen peroxide used was Merck superoxol. This was diluted with conductivity water to give a concentration of about 3 per cent. The actual concentration was determined by the use of 0.1 nitrogen potassium permanganate standardized against sodium oxalate by the method described in TREADWELL and HALL. The peroxide used was brought to a pH of 6.8 by the addition of sodium hydroxide. Brom thymol blue was used as an indicator.

The method of preparing the tissue was standardized as follows: leaves were ground through a food chopper, inserted in washed muslin bags of double thickness, and placed in a hydraulic press. A pressure of 600 units on the pressure gauge was maintained for 1 minute. The juices were collected and to each sample was added 4 cc. of redistilled water for each gram of original leaves used. The mixture was permitted to stand in a constant temperature bath at 20° C. for 1 hour. After stirring the material to insure homogeneity, various amounts were then pipetted out for the determinations.

Table II gives results characteristic of the activity of catalase in leaves with dormant or active notches. Each set of figures repre-

sents an average of two readings, which checked within 5 per cent. Some of the leaves used were dormant, and others active (separated from the plant for 1 week).

The writer's data on catalase activity are parallel with those of BONNS (**6**) on etherization effects on *Gladiolus* bulbs. Catalase activity in germinating seeds has been reported to be greater than in

TABLE II

RELATIVE ACTIVITY IN JUICE EXPRESSED FROM LEAVES OF BRYOPHYLLUM

NOTCH CONDITION	pH	TISSUE SAMPLE (CC.)	H_2O_2 SAMPLE (CC.)	OXYGEN FORMED (CC.)	REACTION TIME (MIN.)	PERCENTAGE DRY WEIGHT
Dormant	5.9	0.5	5	3.8	10	10.42
Active	6.18	0.5	5	3.6	10	11.85
Dormant	5.9	1.0	5	9.33	10	
Active	6.18	1.0	5	10.0	10	
Dormant	5.9	2.0	5	20.4	10	
Active	6.18	2.0	5	20.2	10	
Dormant	5.9	4.0	5	34.1	10	
Active	6.18	4.0	5	32.5	10	
Dormant	5.9	7.0	5	32.8	5	
Active	6.18	7.0	5	32.8	5	
Dormant	5.9	10.0	5	39.4	5	
Active	6.18	10.0	5	34.2	5	

Dry weight determinations were made of tissue samples after chopping in food grinder just before placing in press.

A blank of 5 cc. H_2O_2 for 10 minutes gave a negative reading of 0.5 cc.

similar dormant seeds. Rest-breaking agents are said to stimulate activity in potato tubers. Proliferation in *Bryophyllum* shows no similar relationship.

CARBOHYDRASES

The standard method adopted for these studies was to use, in an Erlenmeyer flask of suitable size, 35 cc. of a 2 per cent solution of carbohydrate with 10 cc. redistilled water and 5 cc. of tissue juice. To each flask toluol was added as a preservative, and the mixtures were placed for 20 hours in a constant temperature darkroom at 24° C. After this period the reducing sugars in each flask were determined.

METHOD OF PREPARING SUBSTRATES.—*Maltose:* 5 gm. maltose hydrate (Pfanstiehl) dried in vacuum oven for 1 hour, plus redistilled water to bring the total volume up to 250 cc., gave the standard 2 per cent solution.

Sucrose: 5 gm. Difco sucrose plus redistilled water to a total volume of 250 cc., gave a 2 per cent solution.

Dextrin: dried in an oven 1 day at 40° C., 5 gm. dextrin (Pfanstiehl) precipitated by alcohol, plus redistilled water to a total volume of 250 cc., gave a 2 per cent solution.

Soluble starch: 5 gm. of soluble starch placed in a flask with 175 cc. redistilled water and boiled for 15 minutes with a reflux condenser; cooled in cold water and washed quantitatively into a 250 cc. volumetric flask. Brought up to volume this procedure gave a 2 per cent solution.

METHOD OF PREPARING TISSUE.—Several leaves were removed from vigorous plants, cut into fourths, and placed in clean glass germinators lined with clean moist filter paper. The sister leaves were each labeled and left attached. After 3 days the leaves showed proliferation from the notches. The excess moisture was removed from the surface by means of blotting paper, and the leaves weighed and ground in a food chopper. This and similar material prepared from the dormant sister leaves was frozen and the juices extracted in a hydraulic press.

METHOD OF DETERMINING REDUCING SUGARS.—The method used is a variation of the SHAFFER and HARTMANN (34) method; the principal change is the dilution of all solutions used to one-fourth the concentration recommended by them.

Table III gives a typical series of results from studies to determine the relative activity of sucrase, maltase, dextrinase, and amylase in leaves with dormant notches or active ones. In this table the value of the reducing sugars in the flasks of each substrate which contained tissue juice from leaves with dormant notches was arbitrarily selected as representing 100 per cent, for the sake of an easy comparison of the value.

When each lot of experimental materials (substrate plus juice) was ready for determination, the material was neutralized with KOH. Each flask of material was then cleared with neutral lead

acetate and deleaded with potassium oxalate. The supernatant liquid was made up to volume, and 20 cc. aliquots were added to, and heated with, Fehling's solutions. Thus, since comparable flasks were treated simultaneously, inactivation of the enzymes of the comparable flasks was accomplished in the normal procedure of determining the reducing sugars present. Negative results reported by BONNS (6) on the activation of carbohydrases in etherized tissues may have been the result of autoclaving the reaction chambers to stop the

TABLE III

RELATIVE ACTIVITY OF VARIOUS CARBOHYDRASES SHOWN BY QUANTITY OF REDUCING SUGARS PRESENT AFTER ACTION OF ENZYMES FOR 20 HOURS

SUBSTRATE	TISSUE TYPE	pH	REDUCING SUGARS IN MG. PER FLASK	REDUCING SUGARS PERCENTAGE COMPARISONS
Sucrose	Dormant	5.05	78.8929	100.00
	Active	5.35	102.0537	129.36
Maltose	Dormant	5.05	202.8184	100.00
	Active	5.35	270.8828	135.55
Dextrin	Dormant	5.05	43.7435	100.00
	Active	5.35	56.8493	129.96
Soluble starch	Dormant	5.05	46.0209	100.00
	Active	5.35	46.8373	101.77

enzyme activity at the end of the reaction period. Masking of the enzyme action by inversion of the substrates at the time of autoclaving is suspected.

From the data in table III it is clear that, under conditions maintained during the experiment, an increase in activity of maltase, sucrase, and dextrinase occurred, but no considerable increase in the activity of amylase was found. It is possible that this enzyme was also activated, but the extended reaction period may have masked differences which would have been apparent at an earlier time.

Chemical relations

The enzyme changes which occur during proliferation should effect a change in the chemical composition of the leaves in which

such proliferation is occurring. Determinations of total carbohydrate, total nitrogen, total phosphorus, and carbohydrate fractions are to be reported. It should be stated, however, that much additional work is required to determine many of the chemical changes which occur concomitantly with activation of foliar meristems of *Bryophyllum*.

METHODS

PREPARATION OF TISSUE.—Leaf tissue to be analyzed was first dried at 80° C. Leaves which had been separated from the plant long enough to show small roots from the notches constituted one lot. Sister leaves which had not been separated from the plant constituted the other lot. Each lot was pulverized in a fine burr mill. The powder thus obtained was placed in glass stoppered weighing bottles, where it was held for future use.

Phosphorus determinations were made according to BRIGG's (**8**) modification of the BELL and DOISEY (**4**) method. Comparisons of color were made by the aid of a Klett colorimeter. Total nitrogen determinations were made by the usual Kjeldahl method modified to include nitrate nitrogen. Total carbohydrates were determined by hydrolyzing powdered leaf tissue for 2 hours in 2.5 per cent HCl in a reflux condenser. Following hydrolysis the material was neutralized, cleared, deleaded, and reducing sugars determined as described. The separation of carbohydrate fractions was as follows.

A. REDUCING SUGARS.—Two gm. of powdered leaf tissue was boiled with 150 cc. of 70 per cent ethyl alcohol for 15 minutes. The mixture was filtered and the filtrate evaporated to dryness and then taken up in water. The resulting solution was cleared and deleaded. The reducing sugars were determined by the modified SHAFFER and HARTMANN method.

B. GLUCOSIDES.—An aliquot of the aqueous solution prepared in A was treated with 2.5 per cent HCl for 5 hours on a boiling water bath. Following neutralization, the solution was brought to volume and the reducing sugars determined.

C. TOTAL SUGARS.—An aliquot part of the aqueous solution prepared in A was hydrolyzed with 2.5 per cent HCl for 15 minutes on a boiling water bath. Following neutralization, the reducing sugars present were determined.

D. DEXTRINS, SOLUBLE STARCH, STARCH.—The residue from filtration in A was strongly boiled with redistilled water for 15 minutes. It was then hydrolyzed with pangestin for 2 hours, during which time it was occasionally stirred. The material was rapidly filtered and the residue saved for E. The filtrate was hydrolyzed with 2.5 per cent HCl for 2.5 hours; then neutralized, cleared, deleaded, and the reducing sugars present determined.

TABLE IV

CHEMICAL COMPOSITION OF COMPARABLE LOTS OF PROLIFERATING AND NON-PROLIFERATING LEAVES OF BRYOPHYLLUM

DETERMINATIONS	PROLIFERATED 20 LEAVES (GM.)	NON-PROLIFERATED 20 LEAVES (GM.)
Original weight	104.1	100.4
Weight when proliferated	60.45	.....
Dry weight	12.00	11.20
Dry weight percentage	8.67	8.96
Total nitrogen	1.24 per 100 gm. dry weight	1.33 per 100 gm. dry weight
Total carbohydrates	54.88 per 100 gm. dry weight	60.82 per 100 gm. dry weight
Total phosphorus	154.94 mg. per 100 gm. dry weight	129.0 mg. per 100 gm. dry weight

E. GUMS, SOLUBLE PECTINS, HEMICELLULOSES.—The residue from filtration in D was hydrolyzed in 2.5 per cent HCl for 5 hours in a reflux condenser; subsequently the material was centrifuged, neutralized, cleared, and deleaded. The reducing sugars were determined.

Twenty leaves from the central and lower regions of plants 1 year old were activated by separation from the plant and by being placed on a stretcher of dry cheese cloth. Sister leaves were tagged on the plants as controls. As soon as growth appeared from the notches of the separated leaves, the two groups were assembled, weighed, and prepared for chemical study. In table IV it will be noted that the proliferating leaves lost approximately 40 per cent of their weight during the period of activation. The data presented in this table show in comparison that in proliferated leaves there are decreases of approximately 10 per cent in carbohydrates, and approximately 6 per cent in nitrogen, and a relative increase of 17 per cent in

phosphorus. It is apparent that no considerable change in carbohydrate/nitrogen ratio necessarily accompanies activation.

To determine whether or not chemical changes in leaves, such as those just noted, may be regarded as the cause of activation of foliar meristems, a study of the chemical constitution of non-proliferating leaves was made. All leaves selected were of an age to permit proliferation. Table V is a summary of the constitution of upper and

TABLE V

CHEMICAL COMPOSITION OF YOUNG AND OLDER NON-PROLIFERATING LEAVES OF BRYOPHYLLUM

DETERMINATIONS	CONSTANT LIGHT		NATURAL LIGHT	
	Upper leaves	Lower leaves	Upper leaves	Lower leaves
Wet weight grams	81.9	77.3	105.1	112.72
Dry weight grams	8.55	12.40	13.20	16.30
Percentage dry weight	10.41	16.04	12.65	14.46
Phosphorus per 100 gm. dry weight	178.93 mg.	80.00 mg.	176.48 mg.	104.17 mg.
Total nitrogen per 100 gm. dry weight	1.6215 gm.	0.7745 gm.	1.5205 gm.	0.8835 gm.
Total carbohydrates per 100 gm. dry weight	21.48 gm.	42.33 gm.	32.83 gm.	42.11 gm.

lower leaves from healthy plants, one lot of which was exposed to constant illumination, whereas the comparable lot received sunlight only.

Between the younger and older leaves of a plant which displays no proliferation, a greater difference in chemical composition is found than between two lots of sister leaves, one of which has active notches and the other dormant ones. However, the differences between proliferating and non-proliferating leaves from the same plant are greater than either the differences between two lots of non-proliferating young leaves, or the differences between two lots of non-proliferating older leaves from separate plants.

In order to force small plants for experimental use, 200 plants 6 weeks old were placed under constant illumination (sunlight supplemented by the light of five 300-watt Mazda bulbs suspended 3 feet above the pots. After an exposure of 12 weeks, a majority of the plants showed small shoots growing from a number of notches.

In not a single case was a root to be found associated with these shoots. Proliferation caused by mutilation usually evidences root growth prior to the formation of shoots.

From a study of the composition of those leaves in constant illumination showing proliferation, contrasted with leaves from plants

TABLE VI

COMPOSITION OF LEAVES OF BRYOPHYLLUM WITH ACTIVE AND DORMANT NOTCHES; CARBOHYDRATE FRACTIONS EXPRESSED AS GM. REDUCING SUGARS PRESENT, FOLLOWING HYDROLYSIS, PER 100 GM. DRY WEIGHT

PRODUCTS	TISSUE TYPE	CARBOHYDRATE PRESENT
Reducing sugars	Active	3.76
	Dormant	2.76
Glucosides	Active	3.98
	Dormant	2.86
Total sugars	Active	4.00
	Dormant	2.64
Dextrins, soluble starch, starch	Active	17.64
	Dormant	26.70
Gums, soluble pectins, and hemicelluloses	Active	3.02
	Dormant	10.26
Total carbohydrates	Active	32.36
	Dormant	45.22
Total nitrogen per 100 gm. dry weight	Active	1.85
	Dormant	1.71

of the same variety and age which had not been exposed to constant illumination, the following relationships were demonstrated: in leaves with active notches there is (1) an increase in reducing sugars, glucosides, and total sugars; (2) a considerable decrease in dextrins, soluble starch, and starch with a reduction of nearly one-third in gums, soluble pectins, and hemicelluloses (table VI).

Discussion

In a complex field of many variables, such as the one presented by the living plant, the concept of limiting factors of the BLACKMAN

(5) type may more completely take into account the kaleidoscopic situations than the concept of a single dominating factor. Indeed, a more favorable approach to a valid explanation of proliferation in many cases seems to be an analysis of factors which limit activation under natural conditions. Especially does this appear to be advisable when it it recalled that such contrasting factors as constant light or constant darkness, drought, or excessively high humidity produce the same end result.

Chemical constitution may be a limiting factor, for under suitable environmental conditions, leaves removed from the upper nodes show activation of notches more than 3 weeks later than leaves removed from the lower nodes. Moreover the oldest leaves of large plants often turn yellow and are finally excised. Repeated attempts to bring about proliferation of these yellowed leaves have been unsuccessful. Immaturity in this case cannot constitute a limiting condition, for younger leaves of the same plant will proliferate.

Changes of osmotic concentration seem important in proliferation, for many of the means of activating the foliar meristems appear to increase the osmotic concentrations in cells adjacent to the meristems, and doubtless of them. Such changes are brought about by water-logging the soil, drought, ringing the stem, cutting the leaf midvein, activation by ether, and stimulation by the action of *Phytomonas tumefaciens*. Furthermore, studies of carbohydrate changes in proliferating leaves display a higher concentration of osmotically active materials in these than in non-proliferating leaves.

Attention is called to the increased activity of hydrolytic enzymes, an accompaniment of proliferation. Substances which have been shown to stimulate enzyme activity also induce foliar proliferation. It is considered probable that, whatever the ultimate cause of proliferation may be, it is the result of new conditions which arise owing to environmental changes or injury.

Conditions which favor activation of foliar meristems may be of a type unfavorable to continued growth of the new organs. An example of such a situation is the activation of notches by drought or ether. Short exposures stimulate activity, but longer ones have a lethal effect. Once new growth is started, it must be supported by

suitable food from one source or another. It is here that LOEB'S mass laws seem to apply with certain limitations. He states:

> Equal masses of sister leaves of *Bryophyllum calycinum* produce in equal time, under similar environmental conditions, approximately equal masses of roots and shoots regardless of the number of roots or shoots formed (except that a more moderate number of shoots formed may possibly permit a more complete utilization of the material furnished by the leaf than if only one shoot is formed).

It is worth while to examine LOEB'S data, to determine the extent to which the mass relation may become a universal statement for the particular phase of proliferation to which it applies. DOSTAL (10) performed a number of experiments with *Scrophularia nodosa*, *Bryophyllum crenatum*, and *Circaea intermedia* which demonstrated that even in *B. crenatum* the relationship does not hold; indeed, departures of approximately 50 per cent from the mass law were reported to occur. Furthermore, the data contain figures in contradiction to LOEB'S statement (23) that "It was found that the mass of shoots regenerated in two sets of halved stems was in exact proportion to the mass of leaves attached to the stems."

In the materials used by DOSTAL, LOEB'S mass relationships do not hold; rather it seems that the smaller the amount of leaf present, the greater the mass of regeneration per gram dry weight of attached leaves.

The possibility of stimulation of notch meristems by many means has been demonstrated. After the production of new plants has been demonstrated by whatever means, the rate of growth of the new structures will be in proportion to the availability of necessary foods, other conditions being similar. The availability of foods would seem to depend on more than the ultimate mass of foods to be drawn upon. The condition of the foods, the relative availability of the proper enzymes, the facility of translocation, and many other factors which are not taken into account by LOEB'S theory, will undoubtedly enter as factors in determining the rate of growth of the newly developing plantlets.

Summary

1. Varietal differences which have been found in *Bryophyllum* may account for many of the contradictions in earlier studies.

2. The meristematic cells of the notches arise at a very early age; in fact, while the leaf is still largely embryonic.

3. A dorsiventral polarity in the proliferating leaf was observed, roots invariably arising abaxially and shoots adaxially in the leaf.

4. The abaxial and adaxial halves of young leaves display a difference in staining reaction to Flemming's triple stain and to certain simple stains. This difference may be correlated with polarity of proliferation.

5. Experimental data are presented which introduce difficulties inexplainable by LOEB's hypotheses of formative stuffs and inhibitive substances.

6. Experiments performed by the writer and by other investigators fail to support two of the fundamental bases of KAKESITA's hypothesis of regeneration.

7. Leaves kept in the dark for a period of 47 days will produce roots and shoots from the marginal notches without subsequent exposure to light. Thus it appears that insufficiency of photosynthate in the leaves before being placed in the dark was not an inhibiting factor as suggested by LOEB.

8. An accumulation of oxidized substances incident to wounding is apparently not an activating agent in proliferation.

9. An apparent correlation exists between conditions which cause a change in carbohydrate synthesis, or in the movement of such photosynthate and proliferation.

10. By quantitative chemical means maltase, sucrase, and dextrinase showed activation amounting to an increase of almost one-third in proliferating leaves over controls of sister leaves.

11. Catalase activity was not particularly increased by conditions which cause activation of foliar meristems.

12. Total phosphorus determined by BRIGGS' modification of the BELL and DOISEY method showed a relative increase of 17 per cent in leaves with active notches compared with sister leaves with resting notches.

13. Quantitative chemical analysis showed that under different conditions total nitrogen may be greater or less in leaves with active notches than in control leaves with resting meristems.

14. Quantitative determinations showed the following relationships existing between the young and older leaves of plants all notches of which are inactive: (1) a difference of 45 per cent in phosphorus content from upper leaves to lower leaves; (2) a difference of 50 per cent in total nitrogen, decreasing from upper to lower leaves; (3) a difference of 97 per cent in total carbohydrates, increasing from younger to older leaves tested.

15. A quantitative comparison of carbohydrates in proliferating leaves with those of similar non-proliferating leaves showed the following differences: in leaves with active meristems there is (1) an increase in reducing sugars, in glucosides, and in total sugars; (2) a considerable decrease in dextrins, and a reduction of nearly one-third in gums, soluble pectins, and hemicelluloses.

16. A reinterpretation and restatement of LOEB'S mass laws have been made on the basis of his data and of data presented by DOSTAL.

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ECOLOGICAL SURVEY OF THE BELLA COOLA REGION

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 419

BLANCHE MCAVOY

(WITH FIVE FIGURES)

Introduction

The Bella Coola Valley is an east and west valley lying wholly in the coast mountains of British Columbia, at the latitude of 52° 21′. It is the landward extension of a series of channels, Fitzhugh, Burke, and North Bentnick, which cut through the Coast Range from the Pacific Ocean north of Vancouver Island. These channels are typical fiords whose grandeur rivals that of the fiords of Norway. They range from one to three miles wide, with nearly parallel sides and with few indentations or constrictions. Their almost perpendicular walls rise from the water's edge precipitously, and in places culminate in snow capped peaks. These mountain sides drop off just as abruptly beneath the surface of the water as they rise sheer above it, and in some places 300 fathoms have been measured and deeper places may occur.

The study recorded in this paper was made during the summers of 1926 and 1928.

GEOLOGY

Concerning the geology of the Coast Mountains, LINDGREN (**6**) states:

> At the end of the Jurassic or the beginning of the lower Cretaceous intrusions began on a large scale along the coast accompanied by marked uplift: magmas of great volume were intruded forming a great chain of batholiths now exposed by erosion all along the Pacific from lower California to Alaska. In comparison with this intrusion all earlier and later phenomena fade into insignificance. The magmas crystallized into coarse granitic rock but its average composition is not that of granite but of a granodiorite. The rocks are uniformly gray or whitish.

SPENCER (**9**) believes that the ranges are remnants of an uplifted and much dissected peneplain that was once continuous with the

belt of interior plateaus. He assigns their elevation to post-Eocene times. DOLMAGE (2) says that the Coast Mountain system consists of batholiths that are 100 miles in width. He states also (3) that in the Bella Coola region Table Mountain and Canoe Crossing Mountain, which are about 30 miles east up the Bella Coola Valley, mark the boundary between the Coast Mountains and the interior plateaus.

In regard to the rivers that move westward through the Coast Mountains, SPENCER (9) is of the opinion that they were antecedent to the uplift of the mountains and have maintained their westward course during the rise of the mountains.

During the Pleistocene the valley glaciers moved westward. Glacial grooves on the south side of North Bentnick arm, not far from the mouth of the Bella Coola River, and grooves at the top of Canoe Crossing Mountain show that the ice moved in a westward direction and was at least one mile deep. DALY (1) says that the Scandinavian region was depressed by the load of ice during the Pleistocene, that both North America and Europe sank under the load of ice, and that since that time the continents have been rising slowly. Small rocky benches along the coast of British Columbia may be evidence of a postglacial rise of the depressed coast.

CLIMATE

Places on or near the coast have their greatest rainfall in the winter, as compared with the summer maximum for places in the interior. Also the coast has an abundant rainfall as compared with the meager rainfall of the interior. Near the coast local conditions may have a great effect on the amount of rain. This effect is shown in the well known differences between Vancouver (58.76 inches) and Capilano Creek (129.46 inches). These two places are less than 10 miles apart. Bella Coola with 46.2 inches and Ocean Falls with 144.21 inches, despite the fact that they are only about 50 miles apart in a direct line, also illustrate this fact. At Bella Coola the greatest rainfall occurs in October and November, with 8 inches as the November average. This heavy rain in the valley, together with the melting of early snow in the mountains, causes a yearly autumn flood in the Bella Coola River.

The average monthly temperatures for Bella Coola vary from 26° F. for January to 61° F. for July for a 10-year interval. At the eastern end of the valley the temperatures are lower in winter and higher in summer than they are at the western end. The amount of rainfall decreases so much at the eastern end of the valley that in some cases gardens are irrigated. No official figures are available.

In summer the Bella Coola Valley is well lighted and the days are long, but in winter there are a few weeks when the sun does not rise high enough to shine over the mountains, and at those times the sun can be seen only when it passes notches in the mountains.

Bella Coola Valley

The Bella Coola River is formed by the junction of the Whitewater and the Atnarko rivers, just east of Table Mountain. From here it flows in a westerly direction for about 40 miles, where it empties into North Bentnick arm. The Whitewater comes from glacial fields, and many of the smaller streams that empty into the Bella Coola River from the south carry large amounts of glacial débris. Like most streams that carry too heavy a load, the Bella Coola River is a braided stream at the western part of the valley. For most of the distance the main channel is close to the foot of the mountains that border the north side of the valley. During the summer months the river is white and thick with glacial silt, but in the winter months the water is clear and less in amount.

The mouth of the Bella Coola River is on the south side of the eastern end of North Bentnick arm, and the Necleetsconney River, coming out of a valley that opens from the north, also empties into the eastern end of North Bentnick arm but north of the mouth of the Bella Coola River.

The water-borne glacial silt of the Bella Coola River is filling in the eastern end of North Bentnick arm, between the mouths of these two rivers, at a rate that has been estimated at about 0.5 foot per year. The tide comes up over the western part of this newly deposited land, which is known locally as the tide flat (fig. 1).

The Bella Coola is a typically U-shaped valley, 1–3 miles wide, hemmed in by mountains that range 4000–10,000 feet high. In some places the floor of the valley has been cleared for farming (fig. 2).

Forests of Bella Coola Valley

WHITFORD and CRAIG (10) state that the valleys that connect the interior plateau with the ocean are a part of the physiographic features of British Columbia that are favorable to economic utilization of forest products. The Bella Coola Valley is one of these favorably located valleys, but up to the present time no widespread cut-

FIG. 1.—Junction of North Bentnick arm and tide flat

tings of the forests have occurred, and much of the valley is in its native condition. This made an ecological survey of the vegetation under natural conditions easily possible.

WHITFORD and CRAIG (10) have mapped the five important forest tree species occurring in the Bella Coola region. These are *Pseudotsuga mucronata*,[1] *Thuja plicata*, *Picea sitchensis*, *Tsuga hetero-*

[1] All plants except *Populus tacamahaca* have been named according to HENRY, J. K., Flora of Southern British Columbia. Specimens have been checked with herbarium specimens in the Field Museum, Chicago.

FIG. 2.—Bella Coola Valley looking eastward and showing cultivated areas

phylla, and *Abies amabilis*. The first four make up the climax forest of the valley floor. The last is not found in the valley but is an important montane species. The climax forest does not occur everywhere in the region, but is common on the floor of the western end of the valley. Undergrowth is not common and the forest floor is covered with a thick growth of *Hylocomium splendens*.

In many places in the coniferous forest there are old trees of *Alnus rubra* and *Populus trichocarpa*. These species are not able to reproduce themselves under the conifers, but are pioneer hangovers in the coniferous forest. The percentages of the different species of conifers in the climax forest are somewhat dependent on the location of the forest in the valley. The nearer the forest is to the tide flat the greater will be the percentage of *Picea sitchensis* and the smaller will be the percentage of *Pseudotsuga mucronata*. The closer the forest is to the southern side of the valley the closer it is to the rim of the mountains that hem in the valley, and the later the lie of snow in spring with a greater percentage of *Tsuga heterophylla*. About 7 miles east of North Bentnick arm a careful count showed 32 per cent of *Thuja plicata*, 21 per cent of *Tsuga heterophylla*, 20 per cent of *Pseudotsuga mucronata*, 19 per cent of *Alnus rubra*, and 8 per cent of *Picea sitchensis*. The trees of such a forest are not close together but the shade is dense. The trees are covered with a heavy growth of mosses and lichens.

Pinus contorta is found as a pioneer on thin rocky soil at the eastern end of the valley. The true subclimax of the region is *Pseudotsuga mucronata*. In addition to its occurrence on the mountains, which will be discussed later, this species occurs in almost pure stands on the floor of the eastern end of the valley. It is also important in the mixed forest. The eastern end of the valley is drier than the western end, and therefore favors the growth of this species. However it is not replacing itself, for under the giant trees of *P. mucronata* the undergrowth consists of *Thuja plicata*, *Tsuga heterophylla*, and *Picea sitchensis*. It is evident that on areas that are now covered with *Pseudotsuga* there will be in the future a mixed coniferous climax forest. *Picea sitchensis* forms a pure stand at the back of the tide flat, and extends for half a mile before it merges into the mixed forest of the valley floor.

Tsuga heterophylla forms a large percentage of the mixed forest along the south side of the valley under the shade of the mountains. Both *T. heterophylla* and *T. mertensiana* grow in solid stand in some of the north and south valleys that join the Bella Coola Valley, especially in the Salloompt Valley where the hemlock forests cover the glacial terraces. Hemlocks are common wherever the snow lies until late in the spring.

In some of the north and south valleys *Abies grandis* occurs with the hemlocks. It occupies the moister and more shaded areas. *Tsuga* in pure stand or with a mixture of *Abies* forms a post-climax in the region. The forest of the mountain sides and the succession of alpine vegetation will be discussed later.

Studies of plant succession

1. Vegetation of tide flat

When the swiftly moving Bella Coola River, heavily laden with glacial silt, meets the incoming tide twice each day at the western end of the valley, the current is slowed down and the water spreads out and drops its load. In this way the tide flat is being built up rapidly, and new land is developing for plants to occupy. Many small streams flowing toward North Bentnick arm are visible only during low tide. Drift wood, brought down the river during times of flood, is scattered all over the tide flat. High tide covers the entire western end of the flat, and no woody vegetation occurs.

In all probability the history of the vegetational development of the tide flat is the same as in all parts of the western end of the Bella Coola Valley. It will, therefore, be interesting to follow the development of vegetation on the flat. A careful study of the vegetation was made on a small island south of the main channel of the Bella Coola River.

The greater part of the western end of the tide flat belongs to a vegetation zone which may be called zone 1. It is an area that is covered longest and deepest by high tide. It extends to the east along the little streams. The plants form tiny hummocks, and between these hummocks the water is a few inches deep even during low tide. The plants are rooted in a white, sticky, silty mud. During low tide the water moves slowly over the flat toward North Bentnick

arm, and during high tide it stands over the area to a depth of several feet. The zone is exposed to the fullest light when the tide is out. The area is dominated by *Hippuris tetraphylla* and *Plantago maritima*. In little ponds on the tide flat *Scirpus validus* may be found, but it is not strictly a plant of the first zone. The pH of the zone ranges from 6.6 to 7.0.

Zone 2 is a rather wide area, lying immediately above the preceding. High tide covers it to a depth of 1.5–2 feet. During low tide

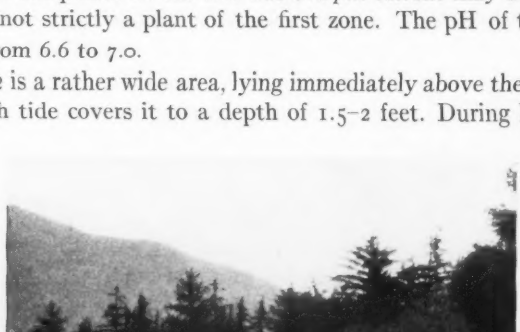

FIG. 3.—Zonation of vegetation back of tide flat

it is water soaked and muddy. It has a grassy aspect and is dominated by *Eleocharis palustris*. *Ranunculus cymbalaria* is abundant, and in some parts of the zone *Potentilla anserina* from the zone above is encroaching upon it.

As the ground gets higher it is barely covered by ordinary high tide and is well drained and comparatively dry at low tide. Zone 3 is a marginal band in front of the first zone of woody plants, dominated by *Carex barbarae*, *Potentilla anserina*, and *Trifolium fimbriatum*.

Only exceptionally high tides that are blown in by western storms go higher than zone 3. Above this area on higher land is zone 4, in

the form of a band of woody shrubs dominated by *Myrica gale*, which range in height from 3 to 4 feet. Under the *Myrica* are herbaceous plants such as *Hordeum nodosum*, *Lathyrus palustris*, *Potentilla anserina*, and mosses.

Immediately behind the *Myrica gale* is zone 5. It consists of a narrow band of *Salix lasiandra*, *S. scouleriana*, and *Lonicera involucrata*. These shrubs and small trees range in height from 8 to 10 feet. There is moss on the ground, but the shade is so dense that herbaceous vegetation is not prominent. Young spruce trees are found occasionally under the willows. Behind the willows is an almost pure stand of *Picea sitchensis*. In some places the trees are so close together that other vegetation is almost completely absent and this species is the sole dominant. The spruce zone extends for almost half a mile before it merges into the mixed coniferous forest of the valley floor (fig. 3).

2. Succession along streams

Since much of the Bella Coola Valley has been made by glacial silt deposited by fresh water, it will be interesting to follow the succession of plants along the streams.

At one place, where 50 m. of new land is known to have been deposited by a stream in the last 25 years, a transect was made so as to extend in a due north and south direction, with the stream at the northern end (fig. 4). When the water of the stream stands at ordinary summer height, a gravelly bank rises to a height of about 0.5 m. above the level of the water. During the periods of summer flood the entire bank is covered with water, and during the fall floods water stands much higher.

Immediately above the bank and paralleling the stream is a belt of young specimens of *Alnus rubra*. This belt is about 3.5 m. wide and extends along the stream for many meters. In places this belt is interrupted. The trees are 1.5–2.5 m. high, with an average height of 2 m. The diameters of the trees are 1–3 cm. The ground on which these trees are growing is somewhat higher than that immediately to the south of it. It is clear that the area occupied by the alders was once a bar, and that the stream flowed at one time through the depression or panne to the south.

The panne is an open area exposed to the full light of the sun. While apparently bare of trees, it is here that the tree seedlings are the thickest. Two species intermingle, *Salix* and *Populus trichocarpa*. No alders are found on any part of the panne (fig. 4). At the

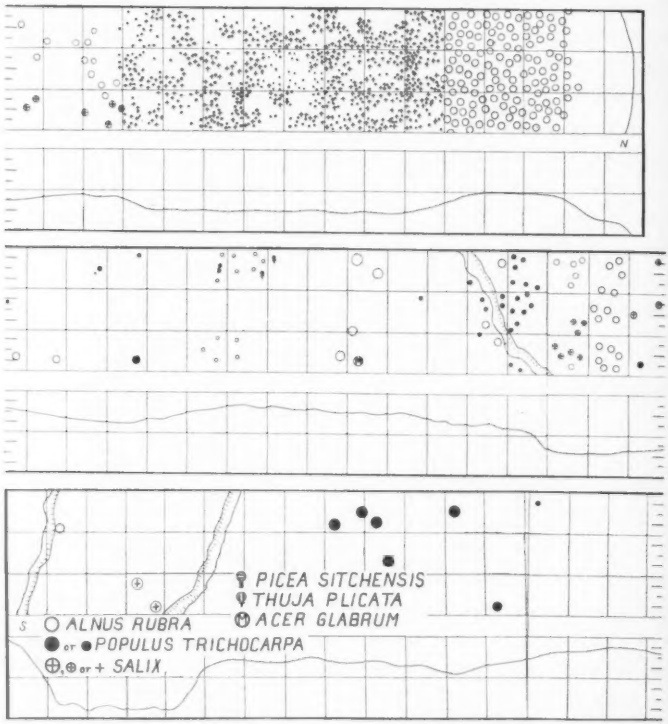

FIG. 4.—Transect across new land made by stream, using 1 m. as unit of measure

present time the panne is beyond the reach of summer floods, but may be covered by the water of fall floods. It is about 15 m. wide, and at the south side the ground rises to a higher level. Near the bank are several small clumps of alders that are about the same size as those along the stream.

The higher land to the south of the panne came into existence as a large island. The trees here are of medium size, scattered, and produce much shade. Many of the largest trees are *Populus tri-*

chocarpa. On the northern side of this land are many medium sized alders. There are a few willows in the depressions, but the trees that are of the most importance from the standpoint of succession are small specimens of *Picea sitchensis* and *Thuja plicata* that are growing in the shade of the larger *Alnus* and *Populus* trees. These indicate that the future vegetation of the area will be a mixed coniferous forest.

At the south side of the area is an old stream bed. The banks on each side of it are rather steep, and on the south bank are old, large alders. In the stream bed are large willows, *Salix lasiandra* and *S. scouleriana*.

It seems proper to draw the conclusion that where new land is formed by stream action, the first trees to grow on areas that are out of the water sufficiently to be well drained are *Alnus rubra*, and on areas that are constantly damp are *Salix* and *Populus trichocarpa*. Moreover, it may be concluded that when sufficient shade is produced to protect the seedlings of *Picea sitchensis* and *Thuja plicata*, these species begin to grow, and if sufficient time is allowed they become the dominant trees of the area. In places that are shaded by the mountains, *Tsuga heterophylla* is found in similar situations.

At the edges of streams which are neither depositing nor cutting to any appreciable degree, shrub vegetation may grow to the very edge. Such shrubs are *Sambucus racemosa*, *Aruncus sylvester*, *Ribes acerifolium*, *Fatsia horrida*, *Rubus spectabilis*, *Cornus stolonifera*, and *Rubus parviflorus*. With the shrubs are large luxuriant clumps of *Asplenium cyclosorum*. Above the water on well rotted logs one can usually find *Phegopteris phegopteris*. With the shrubs are small coniferous trees. Where coniferous trees have had time to get full grown along a stream that has been neither cutting nor depositing through long periods of years, the climax forest conifers grow down to the water's edge. In many places along the Bella Coola and Nutsatsum rivers the species of *Tsuga*, *Thuja*, and *Picea* grow so close to the edge of the stream that branches hang into the water.

3. Successions in muskegs

Beavers have played a large part in the ecological development of the Bella Coola Valley. They have built their dams along the

sloughs and across the smaller streams that enter the Bella Coola River, causing the water to spread out and flood the forested areas. Many of the trees have been cut down or killed and the flooded areas have become well lighted. Back from the centers of the flooding the water has spread out, but it has never been deep enough to kill the trees. They have lived on in a less luxuriant way, and there has been produced a water logged area which is well shaded. Thus beavers have produced two new kinds of areas where two distinct types of plant succession may be found. Light is the main factor that determines the differences in these successions: those in the beaver ponds are primary and those in the water logged areas are secondary.

In the deep water of the beaver ponds are submerged algae and mosses. Where the water is more shallow there are pads of *Nymphaea polysepala*, and in still shallower water large areas covered with *Zannichellia palustris* occur. Closer to the shore there is generally a widespread association of *Equisetum fluviatile*. Animals that range the valley, especially cows, keep the *Equisetum* well cropped, and it is not so abundant now as it was when the settlers first came to this region. In the shallower water the *Equisetum* gives way to *Sparganium multipedunculatum*, and shoreward from that two associations of *Carex* succeed each other. *Carex rostrata* grows in deeper water than does *C. salina*. *C. salina* is found more abundantly around the beaver pond at the western end than it is farther east up the valley.

Around the beaver ponds there is usually developed a muskeg where the following plants are found: *Scirpus microcarpus*, *Carex festiva*, *Glyceria pauciflora*, *Sphagnum* sp., and *Polytrichum* sp.

In the moss, either as islands or on the shore of the ponds, there is a well developed shrub association made up of *Myrica gale*, *Spiraea douglasii*, *Lonicera involucrata*, and *Rubus parviflorus* with *Potentilla palustris*. Occasionally, if there is enough shade, *Lysichiton kamtschatcense* may get started. Under the shrubs are seedlings of the deciduous trees and the conifers that originally occupied the area. Almost always the largest seedlings are those of *Alnus rubra* and *Salix*. *S. lasiandra* and *S. scouleriana* are usually both present. The smaller seedlings are those of *Thuja plicata*, *Picea sitchensis*

and *Tsuga heterophylla*. Back of the shrubs is a narrow association of willows and alders in which are growing coniferous trees. It is easy to see that in a few years the flooded area will be covered with the coniferous forest that is native to the region.

Floating logs in the ponds form centers for plant development. It is on these logs that one may always find *Vaccinium canadense*. The logs are usually covered with a thick growth of moss in which are seedlings of *Picea* and *Thuja*. Where the logs are near the edge of the water and are rather dry, there is a good growth of *Cornus canadensis*. Around the shaded margins of the beaver lakes there is a rank growth of *Lysichiton kamtschatcense*. Where the water has never been deep enough to kill the trees, a shade is cast that is sufficient to prevent the growth of light-demanding species. In such places one plant is always dominant, *Lysichiton kamtschatcense*. It comes in where the water is at least a foot deep, and it is still vigorous where there is no standing water and where the ground is firm enough to walk over. In deep water there are algae in addition to the skunk cabbage. *Sphagnum* helps to build a substratum, and on it *Conocephalum* grows.

In order to determine the frequency of the species in the association that had developed on the shaded and water-logged area (**8, 4**), there were used three thin bars of wood fastened together and arranged so as to fold up and open out. When opened and placed on the ground so as to form three sides of a square meter, the species of plants within the square so formed were noted. By studying a number of sample areas that did not overlap, the percentage of square meters in which each species was found was noted, and these percentages were considered the frequencies of the species in the association.

Such a frequency count gave the following percentages: *Lysichiton kamtschatcense* 96, *Mnium* 60, *Sphagnum* 56, other mosses 48, *Phegopteris dryopteris* 40, *Clintonia uniflora* 40, *Fatsia horrida* 36, *Circaea alpina* 36, *Asplenium cyclosorum* 28, *Hylocomium splendens* 20, *Streptopus amplexifolius* 20, *Alnus rubra* 16, *Equisetum sylvaticum* 12, *Menziesia ferruginea* 12, *Moneses uniflora* 8, *Cornus canadensis* 8, *Picea sitchensis* seedlings 8, *Acer glabrum* seedlings 4, and about four other species in about 4 per cent of all the quadrats ex-

amined. This study shows that *Lysichiton kamtschatcense* and certain mosses are the dominant species in areas that are shaded and water-logged. Where the ground is drier *Fatsia horrida* and the seedlings of the conifers are important.

4. Succession on rock slides

In the Bella Coola region rock slides are common and are important in the base leveling of the mountains. Where the granodiorite of the mountains approaches granite in structure, the slides are made of huge blocks of rocks; but where the mountains are composed of an easily disintegrated rock, the talus slopes consist of small pieces. On the slides made of the huge blocks of rock, lichens are a much more prominent part of the vegetation than they are on the finer talus slopes. There are four or five species of crustose lichens that are commonly found on the rocks of the Bella Coola region.

In places the crustose lichens may be overgrown by a foliose lichen that grows so close to the rock that it is hard to loosen. Sometimes lichens like *Sticta* follow the crustose lichens, and sometimes *Umbilicaria* comes early in the succession. Frequently *Pilophorus cereolus* follows the smallest lichens, being common on all the rock slides of the region. Where succession has gone further there are many kinds of fruticose lichens, including *Stereocaulon paschale*, *S. pruentosum*, *Ramalina farinacea*, *Thamnolia vermicularis*, *Cladonia gracilis*, *C. bellipodophora*, *C. coccifera*, and perhaps others. In time these fruticose lichens are invaded by *Selaginella rupestris*, *Rhytidium robustum*, *Racomitrium canescens*, *Gymnostomum*, and many other mosses. *Hylocomium splendens* is found in a few of the most shaded and mesophytic parts of rock slides, but it is not at all common.

Rooted in the mosses on the rocks are clumps of *Polypodium vulgare* and *Cryptogramma acrostichoides*. About 25 clumps of *Asplenium trichomanes* were discovered, but it may be more abundant. With it is *Lycopodium lucidulum*. *Campanula rotundifolia* is common. Where soil has accumulated, that is, near the bottom of slides, *Pteris aquilina* var. *lanuginosa* and *Aspidium spinulosum* var. *dilatatum* occur. Where shade is afforded, *Vaccinium parviflorum* occurs; where the mosses have built up a sufficient substratum *Linnaea*

borealis may be found. In the crevices between the rocks of a slide the vegetation makes greater progress, and *Pseudotsuga* comes early. Where shade occurs the seedlings of *Thuja plicata*, *Tsuga heterophylla*, and even a few specimens of *Picea sitchensis* may flourish.

On one of the slides at the western end of the valley on a western exposure is a dense growth of *Gaultheria shallon*. It grows in the shade of trees and other shrubs, and is found only on the western exposure of one mountain in the Bella Coola region, although it is common along the channels, especially near Ocean Falls. With this may be found *Vaccinium parviflorum* and *Spiraea discolor*

About 1885, on South Bentnick arm near a mine owned by Mr. F. Jacobsen, there occurred a big rock slide. Mr. Jacobsen said that 40 years later the lower part of the slide was completely grown up to *Thuja plicata*, *Tsuga heterophylla*, *Picea sitchensis*, and *Alnus rubra*, and that the trees were 12–14 inches in diameter.

5. Succession on cut-over land

Clearing in the Bella Coola Valley may be complete or partial, and the degree of clearing is important in determining the type of secondary growth. At the back of the tide flat the spruce forest has been cleared in places to allow grazing space for animals. In some places *Trifolium repens* and *T. fimbriatum* cover the ground. *Agrostis alba* is found with *Trifolium* in some places. Other herbaceous plants in the cleared areas are *Lilium parviflorum*, *Sanguisorba sitchensis*, *Prenanthes alata*, *Trientalis arctica*, *Potentilla anserina*, *Asplenium cyclosorum*. Some parts of the area are becoming overgrown with shrubs, *Rosa nutkana*, *Amelanchier florida*, *Spiraea douglasii*, and *Lonicera involucrata*. Under these shrubs there are seedlings of *Picea sitchensis*. Clumps of half-grown *Picea* are found on all parts of the cleared area at the back of the tide flat.

Where the land was covered with a mixed conifer forest, before it had been cleared for use as pasture land, the plants of the secondary growth represent three general groups, those of the former forest, those of the pasture, and those of the new forest that is establishing itself.

A count on one pasture that is reverting to brush showed that *Rubus viburnifolia* has a frequency of 90, *Poa pratensis* 80, *Populus*

tacamahaca 70, *Trifolium repens* 60, *Cornus stolonifera* 40, *Rubus parviflorus* 35, *Alnus rubra* seedlings 30, *Galium triflorum* 50, *Cerastium vulgatum* 25, *Rumex acetosella* 25, *Sambucus racemosa* 25, *Phegopteris dryopteris* 20, *Ribes divaricatum* 15, *Urtica* 15, *Asplenium cyclosorum* 15, *Spiraea douglasii* 10, *Plantago major* 10, *Picea sitchensis* seedlings 10, *Thuja plicata* 5, *Salix* 15, *Montia sibirica* 5, and *Maianthemum bifolium* var. *kamtschaticum* 5.

The plants of the former forest are in the minority, and are represented by such plants as *Montia sibirica*, *Maianthemum bifolium* var. *kamtschaticum*, and *Phegopteris dryopteris*. The pasture plants are represented by *Poa pratensis* and *Trifolium repens*. The plants which represent the new forest which is in process of becoming established are *Rubus viburnifolia*, *R. parviflorus*, *Populus tacamahaca*, and seedlings of *Alnus rubra*, *Picea sitchensis*, and *Thuja plicata*.

The first plant to invade a pasture which is not being used sufficiently is *Rubus viburnifolia*. Then the seedlings of *Populus tacamahaca* get started and grow into tall trees. In the shade of the deciduous vegetation soon come the seedlings of the conifers *Tsuga*, *Picea*, and *Thuja*. In some places the poplars are 30 feet tall, *Alnus* almost as tall, and the conifers under them about 5 feet high. All this has come on land that was cleared less than 30 years ago, indicating that land becomes reforested rapidly. When the trees have not been cut down in sufficient numbers to clear the ground, and where the partially cleared ground is well shaded, the conifer seedlings that are native to the soil soon replace the trees that were cut.

At the back of the tide flat *Picea sitchensis* seedlings follow on the partially cleared land. Farther east *Picea sitchensis*, *Thuja plicata*, *Tsuga heterophylla*, together with such shrubs as *Sambucus racemosa* soon follow the cutting of the timber. Far up the valley, where the forest is partly cut, seedlings of *Pseudotsuga mucronata* cover the cleared land. In the side valleys, where there is a pure stand of hemlock (*Tsuga heterophylla* and *T. mertensiana*), seedlings of these follow the partial cutting of the timber.

6. Vegetation on burned areas

About 30 years ago there was a severe fire in the Bella Coola region. Since that time there have been no fires of any extent in the

valley, and therefore there was no chance to study the successions that follow recent burns; but present conditions of areas that were burned 30 years ago were studied.

On the mountain side that faces south the burned areas are grown up to an almost pure stand of *Pseudotsuga mucronata*. Where the ground is rocky *Pseudotsuga* is replaced by *Pinus contorta*. The trees are so crowded that some of them are dead, and the shade is so dense that there is little or no vegetation under the trees. The scattered growth consists of *Aralia nudicaulis*, *Arctostaphylos uva-ursi*, *Pyrola minor*, *P. asarifolia*, *Rubus parviflorus*, and a few trees of *Alnus rubra* and *Acer glabrum*.

Where the burned area has a west exposure, under the *Pseudotsuga* occur *Rubus parviflorus* and seedlings of *Tsuga*, *Picea*, and *Thuja*. Conditions on the western exposure are much more mesophytic than on the southern exposure. Where burned areas face north, conditions are very different. Large areas are covered with *Alnus sitchensis*, *Amelanchier alnifolia*, and *Acer glabrum*. Under the deciduous trees are seedlings of *Picea*, *Tsuga*, and *Thuja*. Sometimes both *Tsuga heterophylla* and *T. mertensiana* are found. The deciduous vegetation is much more marked on the north facing mountain than it is on the south facing slopes. At the eastern end of the valley, on a small recently burned area, small *Pseudotsuga* trees cover the ground. On rocky places *Pinus contorta* replaces the *Pseudotsuga*.

Vegetation on eroding surfaces

There are two types of eroding surfaces in the Bella Coola region, glacial terraces in the side valleys that open into the Bella Coola Valley and river terraces along the sloughs through which the flood waters of the Bella Coola River flow during times of flood.

Many of the side valleys were preglacial and filled with gravel at the end of the ice age. Since that time mountain streams have been eroding them, in some places the erosion still occurring. Trees of *Tsuga* are found sliding down such banks, and they grow poorly. The only pioneer tree on such steep slopes is *Alnus rubra*. Such banks are open and exposed to erosion. Along the sloughs the flood waters have cut as many as two or three terraces. *Alnus rubra* constitutes 80 per cent of the tree growth, and *Salix scouleriana* and *S.*

lasiandra 20 per cent. These trees are the pioneers, and under them come *Sambucus racemosa*, *Lonicera involucrata*, *Rubus parviflorus*, and *Symphoricarpus racemosa*. Herbs are not common, *Erigeron philadelphicus* and *Montia sibirica* and in shadier places *Circaea alpina* being found most frequently. *Acer glabrum* and seedlings of *Thuja plicata* and *Picea sitchensis* are found under the willows and alders on the banks.

Mountain vegetation

A detailed study of the vegetation of the mountains of the region and a study of the succession of vegetation on Canoe Crossing Mountain were made. Canoe Crossing Mountain is a part of the northern rim about 20 miles up the valley. It is not a high mountain, being about 1829 m. above sea level. Because of the latitude this is well above the timber line, and affords an excellent study of the alpine and subalpine regions. The vegetation at the top will be discussed first, because all of the vegetation of the mountain has developed since the melting of the glacial ice. The top of Canoe Crossing Mountain has been thoroughly glaciated, as is shown by the glacial grooves, glacial striae, volcanic and other erratics. The glacial grooves show that the valley glacier rode up over the end of the mountain.

1. Snow fields

Near the top of the mountain, especially in sheltered places on the northern and eastern exposures, there are large banks of perpetual snow. This snow is dimpled from exposure to wind and sun. Red snow (*Chlamydomonas nivalis*) is common on these snow fields. This alga extends deeply into the snow and is better developed in the hollows than on the little ridges between the depressions. The snow fields with northern exposure are thoroughly red with the algal growth. The amount of *Chlamydomonas* on the snow with eastern exposure is still less. On snow fields facing west and south there is little or none.

2. Succession in alpine region

Bed rock.—At the top of the mountain there is much bed rock that is exposed to the action of light, wind, and cold. The important crevice plant is *Silene acaulis*. If bed rock is more or less vertical

and well drained, three crustose lichens are the pioneers. They are *Rhizocarpon geographicum*, *Acarospora* sp., and an unidentified lichen. The crustose lichens may be overgrown by a small, black fruticose lichen, *Alectoria divergens*, or by a larger black foliose lichen, *Gyrophora erosa*. Sometimes the latter follows *Alectoria*. On the most exposed parts of the bed rock there is no further stage in the succession.

Where bed rock has been hollowed out by ice, and snow lies until late in the spring, the pioneer is a dark colored moss. *Saxifraga tolmiei* starts in the moss if it is sufficiently wet. Where the moss dries out early in the season *Crocynia* sp., a sorediose lichen, may cover the moss. *S. tolmiei* is followed by *Carex tolmiei*, and it in turn is followed by *Luzula parviflora*, *Juncus subtriflorus*, *Poa alpina*, *Phleum alpinum*, *Trisetum spicatum*, and *Veronica alpina*. In most cases the next association is one of *Spiraea pectinata*, which is followed by *Phyllodoce glandulosa* and *Cassiope mertensiana*. Then follows the climax of the alpine region, *Phyllodoce empetriformis*. With this is *Lycopodium sitchense*. In some of the basin areas the succession goes no further than *Cassiope mertensiana*.

Where the alpine region, which is characterized by the absence of upright trees, merges into the subalpine region with its dwarfed but upright trees, the heather on rocky areas which are exposed to the wind is followed by a poor growth of wind timber made up of *Abies lasiocarpa*, *Pinus albicaulis*, and *Juniperus communis*.

TALUS SLOPES.—Most of the mountain is made up of talus. There may be streams, or the water may spread out into shallow pools. In some places the talus is dry throughout the growing season and in other places it is always wet. Along streams that are deep with rather rocky banks are two pioneers, *Caltha leptosepala* and *Trollius laxus*. *Mimulus alpinus* and *Parnassia fimbriata* sometimes grow with them. On higher ground is *Spiraea pectinata*, which in turn is succeeded by the three heathers with *Phyllodoce empetriformis* and *Lycopodium sitchense* as the climax.

Where the running water spreads out into a thin sheet over the talus, mats of moss fill up the water. *Romanzoffia sitchensis* grows in such wet places. Where the moss is not so boggy *Saxifraga tolmiei* occurs. Boggy places in the talus usually have a dense growth of

vinelike willows, *Salix arctica* and *S. barclayi*, around the edge. Back from such places is the usual succession of *Spiraea pectinata* and the three heathers.

Where the talus slope becomes dry in the early part of the growing season certain mosses are the pioneers. These are followed by a growth of lichens, of which *Crocynia* sp., *Cetraria islandica*, and *Cladonia pyxidata* are the most prominent. Sometimes *Cladonia rangiferina* is found also. *Solorina arctica* and another large green foliose lichen soon overwhelm the smaller lichens and the moss. Usually the series is ended by a dense growth of fruticose lichens, among which may be mentioned *Stereocaulon tomentosum* and a *Cladonia*.

The mosses and the lichens are followed by flowering plants, such as *Saxifraga tolmiei* and *Gentiana propinqua*. In some places the following make a colorful mat, rooted in the moss: *Sibbaldia procumbens*, *Saxifraga bronchialis*, *Pentstemon confertus*, *Pedicularis scopulorum*, *Senecio pauciflorus*, *Oxyria digyna*, *Saxifraga lyallii*, *Pedicularis scopulorum*, *P. bracteosa*, and many others. Often *Campanula rotundifolia* grows in the talus around imbedded large rocks. *Silene acaulis* may sometimes be a pioneer between pieces of talus. Behind the herbaceous associations there always comes *Spiraea pectinata*, followed by the three heathers with *Phyllodoce empetriformis* as the climax.

Some parts of the talus are kept wet throughout the summer by the melting snow. The water is below the surface of the talus and is moving downward all the time. On such places is the best development of the lupine fields. In the wettest places are quantities of *Trollius* also. Several species of *Castilleja* occur, and where it is very wet there may be found creeping willows. Back from such places, where the talus is better drained, occurs the regular succession of *Spiraea pectinata* followed by *Phyllodoce glandulosa*, *Cassiope mertensiana*, and *P. empetriformis*. With the heather there may be *Lycopodium sitchense*.

The pioneers that come in the alpine region depend upon the amount of water and upon the condition of the rocks, but in all parts of the alpine region on Canoe Crossing Mountain, every sere ends with *Spiraea pectinata*, followed by *Phyllodoce glandulosa*, which in

turn is followed by *Cassiope mertensiana* and the climax species *Phyllodoce empetriformis*.

3. Succession in subalpine or Hudsonian zone

At an altitude of about 1585 m. the alpine region merges into the subalpine or Hudsonian zone. This zone extends down the mountain to about 1463 m. Altitudinally it covers about 122 m. On Canoe Crossing Mountain the subalpine zone covers considerable area because the mountain at this altitude is rolling and not at all steep. It has a parklike appearance, with scattered clumps of trees and much low heather. Where the alpine region merges into the subalpine there is a good growth of wind timber, consisting largely of *Abies lasiocarpa* and *Juniperus communis*. Somewhat lower down the trees are upright, but have no branches on the windward side.

Some of the lakes in the subalpine area are deep, with rock bottoms. Around such lakes is *Spiraea pectinata*. In the upper part of the subalpine the *Spiraea* is followed by *Phyllodoce glandulosa*, but in the lower part this is absent and the *Spiraea* is followed by *Cassiope mertensiana*. *Phyllodoce empetriformis* follows *Cassiope*. In the upper part of the zone this species gives way to clumps of trees, mainly *Abies lasiocarpa*, together with a few *Pinus albicaulis*. However, in most of the subalpine area *Phyllodoce empetriformis* is followed by *Rhododendron albiflorum*, *Vaccinium caespitosum*, and *Veratrum viride*. This shrub association is followed by tree growth.

Shallow lakes usually fill up with mats of moss which appear to be rather solid, but which are not safe to walk upon. In this moss, *Caltha leptosepala* and *Trollius laxus* are usually found. *Salix arctica*, *S. barclayi*, and *S. barrattiana* grow around these ponds. They are usually taller on the west sides of such lakes than they are on the east, because the subalpine zone of Canoe Crossing has a western and southern exposure, and the snow on the western side may melt earlier than the snow on the eastern sides. The willows may be vine-like on the east side of such a lake and several feet high on the west side. Around such lakes the succession goes from *Spiraea pectinata* to trees in the order already discussed.

Sometimes the streams that flow through the subalpine region are slow and sluggish. Along such streams are quantities of moss in

which *Caltha* and *Trollius* grow. With them *Saxifraga tolmiei* may be found. Beyond the edges of such boggy places is *Spiraea pectinata*, followed in the regular way by the heathers and *Rhododendron*. In the lowest part of the subalpine area *Pyrus occidentalis* may grow with the *Rhododendron*. These shrubs and small trees are followed by *Abies lasiocarpa*. When streams flow through rocky channels, the pioneers are *Trollius laxus* and *Caltha leptosepala*. *Phyllodoce empetriformis* occupies a large area in this region.

Sometimes the streams are shallow and spread over rocky areas. Here are produced the beautiful wet meadows similar to those in the alpine zone. *Caltha* and *Trollius* are often in the wettest places. Where there is less moisture there are clumps of *Lupinus arcticus* and *Castilleja*. The lupines are succeeded by the heathers and *Veratrum viride*. Where the ground is perfectly level the lupines are often lacking.

In subalpine ravines there is a marked difference on the west facing and east facing sides. The east exposure may be in the condition of the alpine and the west exposure be so thickly covered by alpine firs as to suggest the Canadian or montane zone. The difference is due to the fact that the snow melts more rapidly on the west slope in the early summer. All the firs in the subalpine region, as well as those lower down, are overgrown by *Alectoria fremontii*. In many places there are rocky ledges exposed to the wind where the wind timber grows and the vegetation is alpine rather than subalpine.

4. Vegetation in montane zone

Piper (7) states that the Canadian zone is the most poorly defined zone in the state of Washington. The most characteristic tree in Washington is *Pinus monticola*. *Abies amabilis* and *Tsuga heterophylla*, and sometimes *Thuja plicata*, are also found in the Canadian zone of Washington. On Canoe Crossing Mountain the montane zone extends from about 853 m. to about 1463 m. (fig. 5). The montane zone is made up of three great bands of forests, each occurring as an almost pure stand of a single species. The uppermost band of trees is a forest of *Abies lasiocarpa* with a few scattered specimens of *Pinus albicaulis* and *Tsuga mertensiana*.

Around Mud Lake, which is in the upper part of the montane,

the succession of vegetation is as follows. The open water of the lake is full of a brown floating moss (*Amblystegium*). The first zone

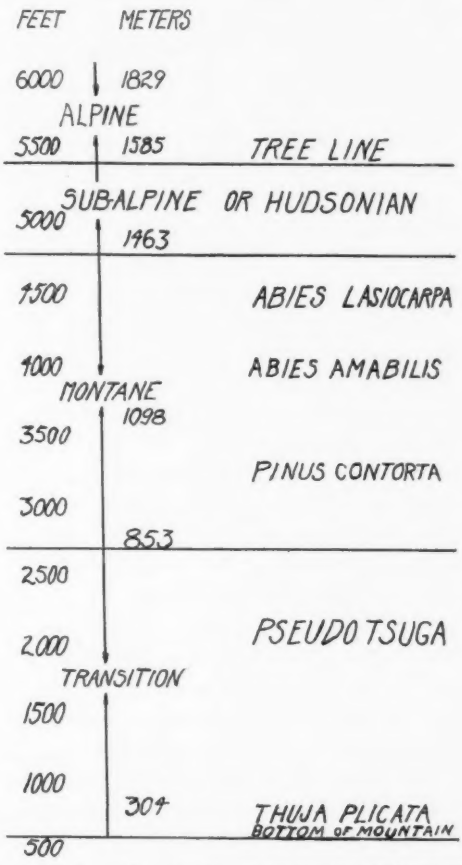

FIG. 5.—Profile of Canoe Crossing Mountain showing dominant vegetation at different altitudes.

of plants rooted in the mud beneath rather deep water is *Carex rostrata*. Closer to the shore is *Eriophorum polystachion*. Shoreward from that is a close growth of *Sphagnum* and other mosses in which

is growing *Carex decidua*. An association of *Carex phaeocephala* follows this. With it is *Poa alpina*.

Out of the reach of water during most of the growing season is a wide and well developed zone extending entirely around the lake, made up of *Spiraea pectinata*, *Luzula divaricata*, *Veratrum viride*, *Valeriana sitchensis*, *Deschampsia atropurpurea*, and several composites.

The two heathers, *Phyllodoce glandulosa* and *Cassiope mertensiana*, which usually follows *Spiraea* in the alpine and subalpine regions, have been eliminated and *Spiraea pectinata* is followed by *Phyllodoce empetriformis* in the upper part of the montane zone. *Phyllodoce empetriformis* was not found below 1310 m. Around Mud Lake the heather is followed by *Rhododendron albiflorum*, *Pyrus occidentalis*, *Veratrum viride*, and *Lupinus arcticus*. This association is succeeded by *Abies lasiocarpa*, growing so densely that there is little or no undergrowth. At about 1310 m. the band of alpine firs gradually merges with a forest of *A. amabilis*. This species forms an almost solid stand down to 1098 m. The lower part of the forest gradually merges into a forest of *Pinus contorta*, which extends from 1098 to 853 m. Most of the trees are 0.3 m. in diameter and are tall and well developed. The pine forest gradually merges into the transition zone below.

5. Transition zone

On Canoe Crossing Mountain much of the soil is made up of small talus material. The slope, although steep, is remarkably free from gullies. There are a few areas of bed rock, but on the mountains west of this elevation are many large areas of exposed rock. There are a few canyons on the mountain side. The lower 200 feet of the mountains that face the south are mesophytic.

Canoe Crossing Mountain begins to rise from the Bella Coola Valley at about 244 m. above sea level. From about 305 m. above sea level the mountain is dominated by *Pseudotsuga*, except on rocky areas. Some of the trees are giants and others are second growth. The lower part of this forest is open and easy to traverse. There is a scattered growth of *Pachystima myrsinites*, *Arctostaphylos uva-ursi*, *Epilobium angustifolium*, *Spiraea discolor*, *Shepherdia canadensis*, and a few other species. The north sides of the trees,

but never the south sides, are covered with a long yellow fruticose lichen. Above 487 m. the undergrowth is much denser, and consists of the preceding species, together with *Pteris aquilina*, *Rubus parviflorus*, and *Cornus canadensis*. Where the shade is thick *Polypodium vulgare*, *Pyrola* sp., and other species occur. Deciduous trees are more in evidence, and *Alnus tenuifolia*, *Amelanchier alnifolia*, and *Acer glabrum* are found. In the more open parts of the forest are young trees of *Pseudotsuga*. In shady parts the undergrowth consists of young trees of *Thuja plicata* and *Tsuga heterophylla*. These species seem to be succeeding *Pseudotsuga*, especially where there is sufficient shade. Often the benches on the sides of the mountain are covered with a very mesophytic forest.

Much of the slope of the south facing mountains is made up of bed rock. Such areas are covered with many kinds of mosses, including *Gymnostomum* sp., *Rhytidium robustum*, *Racomitrium canescens*, and *Mnium insigne*. Almost never does one find *Hylocomium splendens* on the south facing slopes, although it is common on the floor of the valley.

In the mossy areas are patches of *Peltigera*, *Cladonia*, and *Selaginella rupestris*. In summer the patches of mosses have a red brown color and may be seen at great distances. *Cryptogramma acrostichoides* and *Polypodium vulgare*, *Campanula rotundifolia*, *Sedum*, and *Epilobium augustifolium* are also found on the moss covered rocks.

Some of the rocks are covered with shrubs, such as *Pachystima myrsinites*, *Menziesia ferruginea*, *Amelanchier alnifolia*, and *Acer glabrum*. *Pinus contorta* occurs on these rocky areas. Its roots are shallow and able to spread out over the rocks.

Near the bottom of the mountains the forest is very mesophytic. *Pseudotsuga* is mixed with *Thuja plicata*, *Picea sitchensis*, *Alnus rubra*, *Populus trichocarpa*, and *Acer glabrum*. The shrubs of this forest are *Cornus stolonifera*, *Physocarpus*, and *Spiraea discolor*.

The ravines and canyons on the mountain sides are filled with *Fatsia horrida*, *Menziesia ferruginea*, and *Vaccinium ovalifolium*. The tree growth is dense, and consists of *Thuja plicata* and *Acer glabrum*.

6. North facing mountains

The mountains that border the south side of the Bella Coola Valley are much more dissected by small streams and little valleys than are the mountains along the north side of the valley; therefore there are more west, northwest, east, and northeast facing slopes than there are due north exposures. These variations are significant, and are quickly reflected in the species of conifers that are dominant on any particular area.

The west exposure of the lower part of the mountain is covered with a mesophytic forest in which *Thuja plicata* makes up about 35 per cent of the trees, *Tsuga heterophylla* and *T. mertensiana* 20 per cent, *Picea sitchensis* 18 per cent, *Alnus rubra* 10 per cent, *Pseudotsuga mucronata* 8 per cent, *Betula occidentalis* 5 per cent, *Acer glabrum* 3 per cent, and *Populus trichocarpa* 1 per cent. The specimens of *Picea*, *Thuja* and *Tsuga* are large too, but they are not so gigantic as *Pseudotsuga*.

There is dense shade in such a forest and the ground is thickly covered with *Mnium* and *Hylocomium*. The ferns are, *Phegopteris dryopteris*, *Asplenium cyclosorum*, *Aspidium spinulosum*, and *Polystichum munitum*. Such shrubs as *Viburnum pauciflorum*, *Rubus parviflorus*, and *Aruncus sylvester*, and such herbs as *Actaea eburnea*, *Chimaphila umbellata*, and several species of *Pyrola* form a scattered growth.

Well up on one of these mountain slopes with a western exposure is a bench along a mountain stream where there is growing a small group of *Populus tremuloides*. This is the only place in the whole region where this species is known to occur.

Where the mountain faces due north, the forest is made up almost entirely of *Tsuga heterophylla* and *T. mertensiana*. The ground under these trees is completely covered with many kinds of lichens and moss. The most prominent of these are *Peltigera*, *Cladonia*, and *Hylocomium*. Carvings made by the Indians on big boulders and bed rock have been entirely covered with the moss and lichen growth.

On the lower part of the mountain, where large areas of bed rock are exposed, especially where the surface of the rock slopes steeply, *Pinus contorta* is the edaphic dominant. Where the mountain slope

has an east exposure, especially when the side valleys are narrow and the slope is protected from the sun, the forest consists of *Tsuga* intermingled with *Abies grandis*. In such situations the moss is very thick. The snow lies deeply on the ground in the winter and remains until late in the spring. Much moisture is retained in the moss from the snow, rains, and fogs. The *Tsuga-Abies* forest of these east slopes may be considered a post-climax in the region.

No study of the upper slopes of the north facing mountain was made, but residents of the valley who have been on top of these mountains say that the alpine region is covered with the same kinds of heather as are found on the south facing mountains.

Wherever the north facing mountains were burned, some 30 years ago, the slopes have grown up to deciduous vegetation consisting of *Acer glabrum* and *Alnus sitchensis*. Under the trees are halfgrown trees of *Picea sitchensis*, *Thuja plicata*, *Tsuga heterophylla*, and *T. mertensiana*. Such a forest is difficult to traverse, and contrasts strongly with the open *Pseudotsuga* forest on the south facing slopes, which has also grown up since the fire 30 years ago.

Side valleys

The large side valleys that open into the Bella Coola Valley extend in a north and south direction. It is evident that some of them have been preglacial, because they are filled with glacial terraces. Salloompt Valley opens from the north, and its southern end is wide for at least 2.5 miles north of the Bella Coola River. Then the valley becomes narrower and the glacial terraces become prominent. Deep snow lies in the valley in winter, but in early spring the snow melts sooner than it does in the Bella Coola Valley, and for this reason the vegetation of the south end of Salloompt Valley is much more advanced in the spring than vegetation of the Bella Coola Valley.

At the southern end of Salloompt Valley are areas that are grown up to an almost pure stand of *Pseudotsuga*. Farther to the north the forest is more mixed. Large trees of *Picea sitchensis*, *Thuja plicata*, and *Tsuga heterophylla* make up the forest. The undergrowth consists largely of *Tsuga*. Apparently the *Tsuga* forest is a post-climax for the region.

On the terraces the forest consists of *Tsuga* 64 per cent, *Pseudo-*

tsuga 17 per cent, *Thuja plicata* 10 per cent, *Pinus contorta* 2 per cent, *Betula* 1 per cent, *Picea* 4 per cent, and *Alnus rubra* 2 per cent. Openings in the forest are covered with *Pteris aquilina* var. *lanuginosa*. Most such forests have little undergrowth except *Hylocomium*. What there is, is scattered and consists of *Linnaea borealis*, several species of *Vaccinium*, and several kinds of *Pyrola*.

The valleys that open into the Bella Coola Valley from the south retain the snow until late in the spring, and the vegetation is apt to develop later than does vegetation in the Bella Coola Valley. The forests in these shaded and very mesophytic valleys consist largely of *Tsuga heterophylla* and *T. mertensiana*, together with *Abies grandis*. All north and south valleys are characterized by the absence of *Populus trichocarpa*. *Fatsia horrida* is common close to the streams, and in openings.

Floristics

When the continent of North America was cut into two parts by the great Rocky Mountain geosyncline in the Cretaceous period, western North America was a peninsula of Asia, and the eastern part of the continent was attached to Europe. HARSHBERGER (5) states that it was at that time that the eastern and western conifers were assorted and established as distinctly eastern and western species. In the Bella Coola region there is only one species of conifer that is found in the eastern part of the continent. This is *Juniperus communis*, and it is found only in the alpine and subalpine zones, where it occurs as wind timber.

PIPER (7) says that the facts in the distribution of *Picea sitchensis* are peculiar. It is the dominant tree, forming more than 50 per cent of the forest strip along the coast from middle Oregon northward to Kodiak Island, beyond which all timber ceases and the flora becomes that of the Arctic zone. In the north this species ends sharply in the Arctic meadows, and in the south it merges gradually with the *Pseudotsuga* forests. This enormous stretch of a single species at sea level is probably due to the remarkably equable temperature and great humidity of the immediate sea coast. According to WHITFORD and CRAIG (10), *Picea sitchensis* is confined to the Pacific Coast, seldom extending back farther than 50 miles from the salt water. It seldom occurs in a pure stand, and is a prolific seeder.

Abies grandis occurs rarely in the Bella Coola region. It is found in the side valleys that open into the Bella Coola Valley from the south. These valleys are closely shaded by the mountains and open to the north, and they are deeply filled with snow until late in the spring. *Abies grandis* does not occur in a pure stand, but is always found growing with *Tsuga heterophylla* and *T. mertensiana*. Mr. F. Jacobsen, who lives in the valley, says that *Chamaecyparis nootkatensis* is found in only one place in the Bella Coola region, and that is far up in one of the mountains along the northern border at the western end of the valley.

There are only two pines found in the area studied. *Pinus albicaulis* grows in the alpine and subalpine zones but not abundantly. *P. contorta* grows as an edaphic pioneer on rocky places, and is found abundantly in the montane zone. *Pseudotsuga mucronata* evidently has come into the Bella Coola Valley from the interior plateau, and is found at its best at the eastern end, where the climate is more continental than it is nearer the coast.

Both *Tsuga heterophylla* and *T. mertensiana* grow together in the valley and on the north facing slopes. In the valley bottom *T. heterophylla* is much the commoner of the two in the mixed forest.

The poplars of the region are interesting. *Populus trichocarpa* is the common species. It is a pioneer on damp ground along streams, and is almost the ecological equivalent of the willows in the seedling condition, but in the climax forest the poplars are present as hangovers because they are long-lived trees. *P. trichocarpa* is seldom or never found in any of the north and south valleys. For its establishment as a seedling it needs strong light as well as moisture. Sufficient light is not available in north and south valleys. *Populus tacamahaca* is found rarely in the valley. It is a pioneer on cutover land that has been pastured. *P. tremuloides* is found in but one place in the Bella Coola region. It grows on one of the mountains that borders the south side of the valley, along a mountain stream.

There are two willows in the valley and three in the alpine and subalpine parts of the mountains. The two species of low altitude are *Salix lasiandra* and *S. scouleriana*. The three species of the mountain tops are *S. arctica*, *S. barrattiana*, and *S. barclayi*.

The ferns of the region are cosmopolitan. *Asplenium trichomanes* grows in about 25 clumps on the large rock slide at the western end of the valley. Only this station was found for it. *Botrychium simplex* and *B. ramosum* were found in one location, but since they are so small they may have been overlooked in other places.

Many species of heaths are common, but the distribution of *Gaultheria shallon* is the most outstanding of all. It is found in only one place in the region. It grows on the west exposure of a mountain at the western end of the valley along the northern rim. Here it occurs in the shade of the coniferous forest, and makes traveling through the forest difficult. This very local growth, as contrasted with its common and widespread occurrence along the channels farther west, is noteworthy.

Lichens are abundant everywhere. They cover the fence posts, tree trunks, tree limbs, and rocks from sea level to the mountain tops. No *Fucus* is found in the waters of North Bentnick arm near the mouth of the Bella Coola River. This species is common along the coast, and its absence here is noteworthy and is probably due to the dilution of the saline waters of North Bentnick by the fresh water of the Bella Coola River.

This work was done under the direction of Dr. HENRY C. COWLES, and to him I wish to express thanks for advice and for the loan of certain instruments. I am also indebted to Dr. GEORGE D. FULLER for much valuable help and for the loan of instruments, to Dr. BRUCE FINK and to Dr. CHARLES PLITT for identifying the lichens, to Dr. JOHN DAVIDSON for identifying certain of the herbaceous species, and to Miss MILDRED IRWIN for identifying certain of the mosses.

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GERMINATION OF SPORES AND DEVELOPMENT OF JUVENILE THALLUS OF RIELLA AMERICANA

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 420

R. A. STUDHALTER

(WITH TWENTY-FIVE FIGURES)

Introduction

Botanists have long been interested in *Riella*, a genus of submerged aquatic liverworts, because of its peculiar shape. The adult plant consists of a thickened stem, on one side of and over the tip of which is a wing one cell in thickness. Rhizoids develop near the base of the stem; archegonia are produced along its margin; and antheridia develop along the outer edge of the wing, which is here somewhat thickened. The origin and morphological interpretation of the wing have been subjects of much controversy.

The first known species of *Riella* was called *Sphaerocarpus notarisii*, and was described from Sardinia in 1839. With the discovery of a new form in Algeria, the two species were placed together into the new genus *Duriaea* in 1843. In 1852, MONTAGNE added a third species from Switzerland to the group, and changed the name to *Riella* on account of the previous use of the name *Duriaea* for a genus of the Umbelliferae. Eleven species are now known, from Algeria, Sardinia and Greece, Switzerland, France, Turkestan, South Africa, the Canary Islands, and Texas. The distribution of each is more or less local, some having been collected only a few times; and one species has not been seen in its native habitat, having been seen only in culture from mud collected in Turkestan (11).

Riella americana was described in 1903 by HOWE and UNDERWOOD (9) from specimens collected in western Texas. This, together with a fragmentary specimen from the same place in 1855, and another fragmentary specimen from South Dakota in 1898, are the only recorded collections of the species until its rediscovery by the writer

in the spring of 1927. The work of HOWE and UNDERWOOD is apparently the only published paper on the species.

Materials and methods

The spores used in the germination studies herein reported were obtained in western Texas at various times from 1928 to 1930. Plants with mature sporophytes were collected, together with some of the sand, from the bottom of the creek in which they were growing. In most instances this mixture was permitted to air-dry in the laboratory, but in other cases it was kept in water in a closed jar until used.

For purposes of germination, some of this material was placed during the warmer months of the year in a moist chamber or jar in 2–3 inches of distilled water and placed on window sills where direct sunlight was available part of the day. In other instances the cultures were heated to temperatures ranging from 40° to 75° C. for one or two hours before being placed in the window. The evaporated water was replaced with distilled water, and occasionally a mixture of distilled and tap water. The presence of the sand from the natural habitat is believed to have furnished a partially natural condition for the material.

For the purpose of study, some of the sediment in the bottom of the culture was drawn off with a wide mouthed pipette. Every few days a portion of the material was preserved in formalin-alcohol for future comparison with the fresh green sporelings, the latter serving as the basis for most of the study as well as for the drawings.

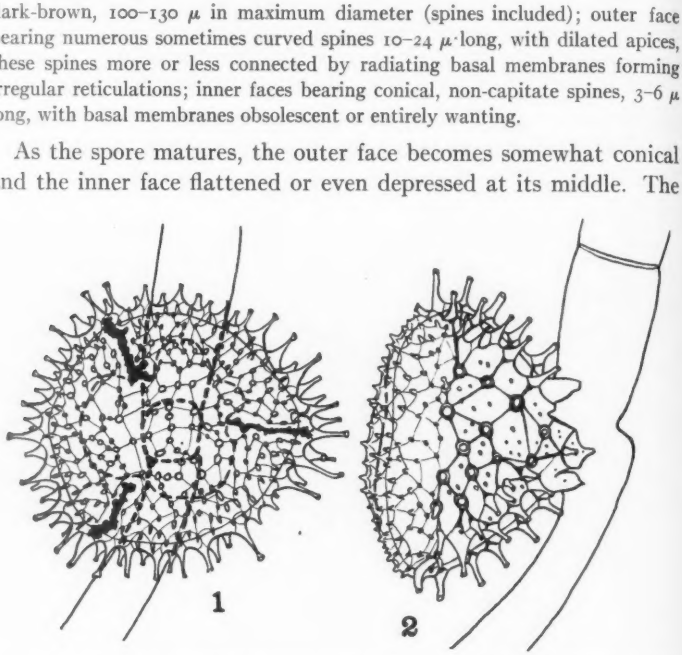
The more elaborate methods used by other workers in germinating the spores of *Riella*, often in addition to simple water cultures, were not found necessary for *R. americana*. PORSILD (12) used mud, filter paper, loess, or kaolin covered with water. HOWE and UNDERWOOD (9) used filter paper. GOEBEL (6) also used kaolin. It is believed that pipetting the sporelings is less injurious to them than picking them off of a more solid substrate; but this method has the disadvantage of making it more difficult to obtain a clear idea of the orientation of the sporelings in the water, and of disturbing this orientation more frequently.

First germination stages

The spores of *Riella americana* are described by HOWE and UNDERWOOD (9) as follows:

> dark-brown, 100–130 μ in maximum diameter (spines included); outer face bearing numerous sometimes curved spines 10–24 μ long, with dilated apices, these spines more or less connected by radiating basal membranes forming irregular reticulations; inner faces bearing conical, non-capitate spines, 3–6 μ long, with basal membranes obsolescent or entirely wanting.

As the spore matures, the outer face becomes somewhat conical and the inner face flattened or even depressed at its middle. The



FIGS. 1, 2.—Fig. 1, germinating spore from inner face; long capitate spines of outer face extend over margin; irregular radial ridges shown in solid black, boundary of central depression dotted; fig. 2, germinating spore from side; germ tube emerging from middle of outer and more spiny face; ×420.

triradial ridges are usually completely obliterated, and, if seen at all, are merely rough, irregular, dark ridges toward the outer portion of the inner face (figs. 1, 2).

The spores of *R. americana* are found to germinate better after a period of rest. Fresh spores, apparently mature, give a low percentage of germination; while spores from the same material up to 17 months of age (the oldest available) which had been kept dry or in water in the dark, germinated in excess of 50 per cent in a few

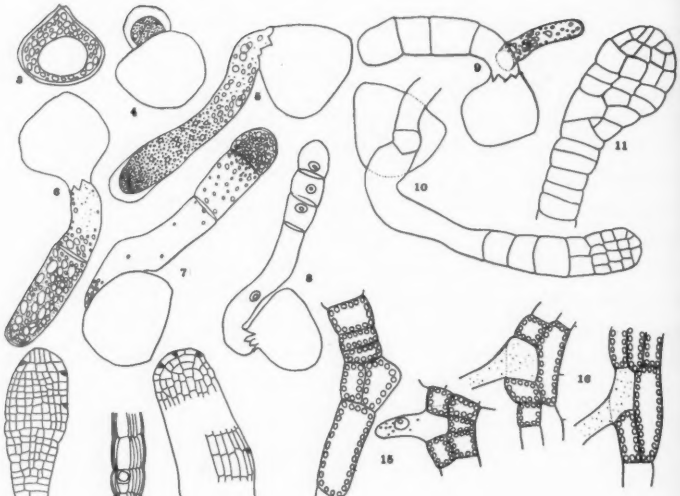
days. Heating the spores in water before the culture was made was found to hasten germination of air-dried spores recently collected, but often to be detrimental to older spores. Strong sunlight was found advantageous in the early stages of germination.

The spores of other species of *Riella* also require a rest period after apparent maturity. Germination resulted for HOWE and UNDERWOOD working with *R. americana* in the case of only a few of the spores collected several months previously. The same workers obtained more than 50 per cent germination of spores of *R. affinis* in a few days from specimens which had been in the herbarium for five and a half years. Spores of *R. paulsenii* germinated for PORSILD (**11**, **12**) from mud collected about three years previously, and CAVERS (**2**) obtained germination of *R. capensis* from dried mud six years old.

In 1912, Dr. C. J. CHAMBERLAIN received from South Africa some dry soil from which a culture was made. Algae developed but no *Riella* made its appearance. *R. capensis* appeared very abundantly in a second culture, however, made from the same soil in 1925, 13 years after collection. Hundreds of plants were carried to the fruiting stage. This information was transmitted verbally by Dr. CHAMBERLAIN, with permission to quote it.

In several instances intracapsular germination was observed for the Texas species. Many of the spores germinated simultaneously, the germ tubes and rhizoids readily breaking through the slightly decomposed sporangium wall. Such germination was also seen by PORSILD (**12**) for *R. paulsenii*, and is mentioned by DOUIN (**4**) and GOEBEL (**7**).

There is little or no swelling of the spore of *R. americana* during germination; it remains essentially the same size and shape, and the depression at the middle of the inner face does not straighten or bulge out (fig. 2). Within two or three days after dried spores are placed under conditions favorable for germination, the wall becomes slightly more transparent and oil drops of various sizes are seen to occupy a large part of the interior of the spore (fig. 3). Just before rupture of the spore wall, a single large vacuole is formed opposite the middle of the inner face (fig. 3). The nucleus is not visible in unstained spores. The time required for the rupture varies from 4



FIGS. 3–19.—Fig. 3, outline of spore with wall about to rupture; fig. 4, germ tube emerging from under side of spore; fig. 5, sporeling before formation of first cross wall; fig. 6, sporeling after formation of first cross wall; fig. 7, after formation of second cross wall (all ×165). Fig. 8, sporeling after formation of third cross wall, showing positions of nuclei; ×140. Fig. 9, sporeling showing origin of primary rhizoid; distal cell slightly green, other two yellowish; fig. 10, division of distal cells to form early stage of primary thallus; all cells green; primary rhizoid three times length of germ tube; fig. 11, further divisions of distal cells of filament; ×165. Fig. 12, later stage of primary thallus; ×115. Fig. 13, portion of primary thallus seen from edge, with two large oil cells; ×165. Fig. 14, mature primary thallus; ×75. Fig. 15, two stages of origin of secondary rhizoid; fig. 16, two figures showing relation of secondary rhizoid to mother cell and surrounding cells; ×165. Fig. 17, intercalary apical cell at base of expanded portion of primary thallus; fig. 18, early stage of lateral intercalary outgrowth; ×165. Fig. 19, primary thallus showing lateral outgrowth on both sides; twin plants result in such cases; ×37.

to 18 days, the shorter periods being more common. The rupture takes place at the middle of the outer or more spiny face (fig. 2), which is the apex of a rounded pyramid. This point of rupture, previously reported by HOWE and UNDERWOOD, is the exception for the spores of bryophytes and pteridophytes, which usually germinate at the middle of the inner face, that is, at the junction of the radial cracks or ridges. Possibly this condition in *R. americana* is associated with the shape of the spore, the apex of the pyramid on the outer face representing the weakest part of the spore wall. Or, possibly it is a hold-over from an ancestral condition in which the spores of a tetrad cling together after maturity, as do those of *Sphaerocarpus* (CAMPBELL 1). The rupture is effected by portions of the spore wall being lifted away from the apex and raised more or less at right angles to the spore. The resulting opening is characteristically circular (fig. 1); occasionally triangular, square, or irregularly angled openings are found, but always with the two dimensions roughly equal.

The first structure to break through is the germ tube (fig. 4), although in rare instances the primary rhizoid makes its appearance first. The germ tube at first grows out straight from the spore, but usually before it reaches a distance of 100 μ it bends to one side (fig. 5). This is no doubt a reaction to the stimulus of light or gravity, for occasionally growth is straight outward, and at other times the tube follows the spore wall half way around the spore before growing away from it. The end wall of the tube is at first somewhat thick (fig. 4), but later is no thicker than the side walls. The germ tube reaches a length of 80–150 μ before the first cross wall is formed (fig. 6); under conditions of weak illumination a length of 500 or even 700 μ has been observed. The diameter of the germ tube is nearly uniform throughout, ranging from 26 to 44 μ with an average of 33 μ. Generally, however, it is somewhat narrow at the spore and slightly enlarged at the tip.

The contents of the germ tube consist largely of closely packed oil drops (fig. 6). Small granules are also visible. As the germ tube grows, the oil drops become smaller and more scattered, remaining most numerous toward the tip of the tube. Chloroplasts and rhizoids are not seen at this stage.

HOWE and UNDERWOOD were the first to report on the early stages of germination of any species of *Riella*. In both *R. americana* and *R. affinis* they found the germ tube emerging before the rhizoid; in the former species it always developed from the outer face near its middle, and in the latter the same was true in practically all cases. The shape of the opening in the spore wall is not described nor shown in the drawings. PORSILD (12) found that the spore of *R. paulsenii* was cracked open along a straight median line more than half way around the spore. He found that a simple germ tube was formed, the length and width of which are dependent upon light, being short and broad in well lighted cultures on filter paper or kaolin. DOUIN (4) shows, without comment, a sporeling of *R. clausonis* in which the spore is split open along a straight median line. Other investigators give no details whatever on this stage, or even fail to mention it.

Filamentous stage

At the time of formation of the first cross wall, 45–115 μ from the spore, the oil drops are more numerous toward the tip of the filament, and no chloroplasts are visible. Growth of the filament continues at its tip, which remains rounded, and soon a second cross wall is formed (fig. 7) about midway between the first one and the tip. Growth continues in this manner until four to seven cells are formed in a linear series (fig. 8). These cells have an average length of 35 μ and an average diameter of 33 μ. Even in those cases in which the filament had become enormously elongated before being divided by cross walls, the new cells formed were of the sizes indicated.

As growth continues, the oil drops become smaller and gradually disappear; chloroplasts make their appearance, taking their place (fig. 9). The plastids are sometimes found in the 1-celled stage, but more frequently are not seen until two or three cross walls have been formed. The terminal cell is the first to become yellow, and then green, and the greening proceeds cell by cell toward the spore. In no case were plastids seen between the spore and the nearest cross wall, this region of the germ tube remaining colorless and containing scattered small oil drops and granules.

The primary rhizoid is generally formed after the second or third cross wall has been laid down (fig. 9). It makes its appearance on

the convex side of the germ tube, between the spore and the nearest cross wall, close to or directly at the spore. From a circular area a globular protrusion is formed, somewhat smaller in diameter than the germ tube, and the rhizoid thus formed protrudes farther as a tubelike outgrowth, which bends back over the spore and grows in a direction opposite to that of the germ tube. It is a cylindrical, smooth walled structure of uniform diameter (17–28 μ, average 20 μ), with a rounded, thickened end wall. Its lumen is continuous with that of the base of the germ tube. Only in rare instances was a single cross wall observed, always some distance from the spore. The protoplasm remains granular and is at first densely crowded with oil drops, which become scattered as the rhizoid increases in length, but which remain more numerous near the tip. The nucleus is found a short distance back from the tip.

The primary rhizoid elongates rapidly, while the filament slackens its rate of growth so that the rhizoid is soon much longer than the germ tube. No secondary rhizoids have been observed at this stage. The growth of this stage is completed in two or three days.

HOFMEISTER (**8**) states that in *Riella reuteri* a short filament is formed upon germination, but he presents no description nor figures. TRABUT (**16**) says nothing about the early stages of development of *R. cossoniana*, but he shows two figures (without indicating cells) which he calls the protonema, but which, as pointed out by HOWE and UNDERWOOD, are more likely plants originating from gemmae. HOWE and UNDERWOOD state that in *R. americana* the germ tube is divided by transverse walls into a single row of several cells. More detail is given by these writers for *R. affinis*, in which the first wall was found to be somewhat curved, with its convexity turned toward the spore. The part above contained most of the starch grains, and in the course of time began to show chlorophyll. The length of the germ tube from the spore wall to the curved septum was found to vary from 0.02 to 0.7 mm. SOLMS (**13**) states merely that a filament of cells is produced by *R. parisii*. PORSILD (**12**) found that in *R. paulsenii* the germ tube becomes divided immediately by cross walls, and that the first rhizoid usually develops at the first cross wall.

Primary thallus stage

A few days after germination, when the filament has reached four to seven cells in length, its end broadens and becomes rounder. The exact order of wall formation was not observed, but in a short time the end cell has divided longitudinally and transversely into several small cells, all in the same plane, forming a rounded flattened structure one cell in thickness (fig. 10). Soon the second cell from the tip undergoes similar divisions, then the third, etc., in basipetal succession (fig. 11). The result is that the cells are smaller and younger at the tip, and progressively larger and older toward the spore. All oil drops disappear, and the peripheral regions of each cell become closely packed with chloroplasts.

For a time the derivatives of each of the original cells can be followed distinctly (fig. 12). The new thallus is at first broader at its tip, and later broader at or just below its middle. The thallus at this stage is usually less than 1 mm. in length at its maximum, but one thallus 1.5 mm. long was observed. Its width varies up to 0.5 mm. (usually less), and up to 30 cells at its widest part. Its shape varies from almost circular to egg-shaped, elongated oval, or much elongated. Division is in two planes, so that the thallus remains one cell in thickness, although exceptions have been found rarely in which the middle basal region is two cells in thickness. The thallus is somewhat lens-shaped in cross-section; the marginal cells are 13–18 μ thick, and the cells become progressively thicker toward the center of the thallus, where they have the thickness of the original cells of the filament, 35 μ (fig. 13).

No apical cell has been observed in the thallus at this stage. The cells at the tip remain smaller and more active at first, but soon the thallus becomes meristematic throughout. As the thallus approaches its maximum size at this stage, a zone of cells extending across the base of the broadened portion remains more meristematic, and the cells above this may be slightly elongated.

Sometimes all of the cells of the germ tube are involved in the broadening of the thallus; more commonly one, and less frequently two, three, or four nearest the spore remain undivided for a time (fig. 12). With maturity of the thallus this proximal group of cells divides further in two planes to form a zone one cell thick, one to

four cells broad (usually two), and one to eight cells long (usually four to six). The cell nearest the spore may remain undivided. These cells become much elongated, and are no doubt efficient in the conduction of materials absorbed by the rhizoids (fig. 14).

Oil cells begin to form on the expanded portion of the thallus when this region is six to eight cells broad (fig. 12). They are formed almost exclusively in the marginal row of cells, certain of which cut off a small cell from the end or outer corner. Such a small cell is prevailingly triangular, although frequently wedge-shaped or rectangular. Shortly after its formation, a large oil drop of irregular outline appears within it, and often almost fills it. Although surrounding cells may continue to divide, oil cells are not capable of further division.

The primary rhizoid continues to elongate until it is two to six times the length of the plant from spore to tip of thallus.

Secondary rhizoids take their origin from the elongated cells below the meristematic zone. The first ones appear nearest the spore, and the later ones progressively at more distal points; none has been seen to arise from the meristematic zone or from the regions beyond. In number they vary up to eight. Their method of formation is characteristic. The proximal portion of a lateral wall of a cell bulges out and forms an outgrowth which becomes a cylinder of uniform diameter (fig. 15). The chloroplasts are lost early, and both cell and rhizoid remain colorless (fig. 16). The secondary rhizoids have the same diameter as the primary rhizoid, and soon approach its length. They also have a thickened, rounded end wall, are smooth, of uniform diameter, and have no cross walls. As the chloroplasts are lost, scattered oil drops appear, the protoplasm becomes granular, especially at the tip, and the nucleus is found a short distance back from it (fig. 16).

When the primary thallus has reached its maximum size, therefore, the plant consists of the following parts: the spore; the primary rhizoid, which extends in one direction; the base of the original germ tube, which extends in the opposite direction; a long narrow band of elongated cells from some of which extend the secondary rhizoids; and a broadened unistratose region containing oil cells at its margin and remaining meristematic at its base (fig. 14). The

growth of the primary thallus stage up to this point was found to require two to three weeks. The empty spore wall remains attached until the primary thallus is mature. By the time it becomes broken off it appears to be partially decomposed, is swollen, and is more transparent than before germination. Strong sunlight is often detrimental to the thallus after the filamentous stage, rendering it stunted and broad. In weak light the thallus may be long and ribbon-shaped.

Among the abnormal shapes observed were those in which the distal portion of the thallus was twisted at a sharp angle to the proximal portion, the median line of the two portions being continuous and straight. This was probably due to a change of orientation with regard to light.

Many observations have been made by other workers on the primary thallus stage of various species of *Riella*. On the whole their interpretations agree, the main controversy being centered on the presence or absence of an apical cell. That an apical cell is present near the tip of the thallus was believed by HOFMEISTER (**8**), and LEITGEB (**10**) did not consider the growing region intercalary. HOWE and UNDERWOOD (**9**), GOEBEL (**5**), SOLMS (**13, 14**), and PORSILD (**12**) find no apical cell, but instead either consider the entire broadened portion of the thallus meristematic, or else recognize an intercalary meristematic region. DOUIN (**4**) figures the primary thallus stage without showing cells, and without description. The latter investigator, as well as the others, gives descriptions of the primary thallus of the several species which correspond rather closely to that of *R. americana*.

Lateral outgrowth and differentiation of stem and wing

When the cultures were three to five weeks old, growth became localized at one or both sides of the margin of the intercalary region of growth. Usually the cells on one side and at the middle mature, while those at the opposite margin remain meristematic. If only the middle cells mature and both margins remain meristematic, however, twin plants result (fig. 19).

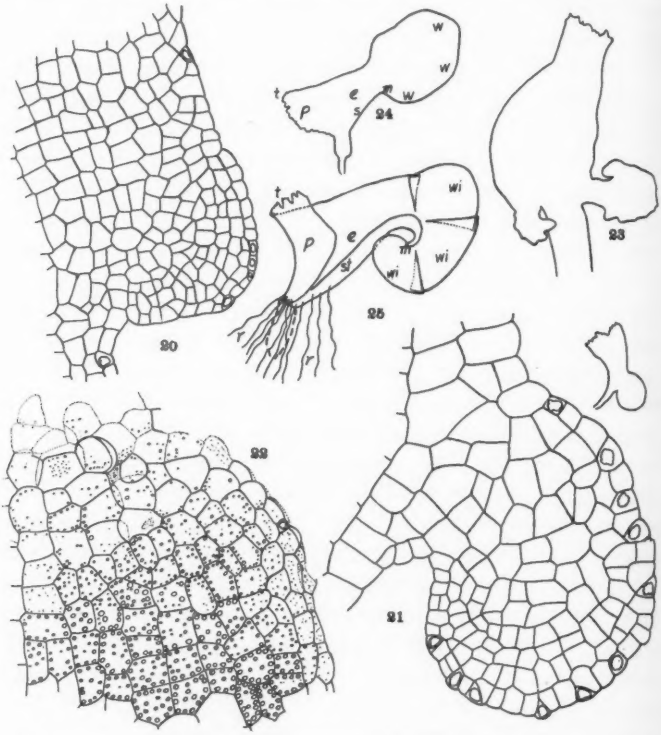
There is generally no indication of an apical cell along the meristematic margin, but sometimes a single large cell with small plastids, close to one or two oil cells, functions as an apical cell (fig. 17).

Even in these instances, however, the condition is purely temporary, the apical cell soon giving way to a number of small, actively dividing cells, which constitute a more permanent growing region (fig. 18). The cells of this region are the smallest ones of the thallus, except the oil cells. The smallest and most active of the meristematic group are those closest to the base of the thallus, that is, nearest the median line (proximal with reference to the spore). Walls come in irregularly at any angle, so that orientation of one cell bears no definite relation to that of any other, except that all cells lie in the same plane. The marginal cells, however, form a more definite row.

The new mass of tissue grows downward and outward so as to form an angle of 45°–60° with the elongated cells below the meristematic zone, and it maintains throughout a position in the same plane as the primary thallus. A semicircular mass of tissue is soon formed (figs. 19, 20), which later becomes an almost circular outgrowth one cell in thickness (fig. 21). The cells of the adjacent primary thallus become somewhat distorted. As lateral growth continues, the primary thallus is bent to one side until it may occupy a position at right angles to its original axis, and the new growth also extends out more nearly at right angles to the original axis of the thallus (fig. 21). The cells at the base of the new growth elongate and carry the meristematic zone some distance from the primary thallus. At the same time an overgrowth occurs just beyond, on the proximal side (that is, toward the spore). This overgrowth extends around the tip of the meristematic region and back toward the primary thallus, nearly parallel with the axis of the new outgrowth, on its distal side (away from the spore). This results in the lateral outgrowth having a falcate structure which is similar, in miniature, to the shape of the adult plant, the most active cells being in the notch of the overgrown portion, and the cells between the notch and the primary thallus showing a tendency toward elongation (figs. 24, 25). Oil cells at first are not so numerous in the region of active growth, but appear on the margin of the falcate area some distance from the growing zone.

During progress of the lateral outgrowth, the primary thallus undergoes further changes. The cells at its tip lose their chloroplasts, protoplasmic granules become fewer, the cells separate at the cor-

ners and sides and come to overlap each other, their lumina finally become empty, and the walls break down and become detached



FIGS. 20–25.—Fig. 20, later stage of lateral outgrowth; ×145. Fig. 21, circular stage of lateral outgrowth; primary thallus bent to one side and partially resorbed; large figure ×210. Fig. 22, resorption at tip of primary thallus; ×145. Fig. 23, abnormal secondary development of primary thalli from original primary thallus (one shows single lateral growing region, the other shows two); fig. 24, juvenile thallus shortly before differentiation of stem and wing; ×32. Fig. 25, juvenile thallus after stem and wing have become differentiated (diagrammatic): *e*, region of elongated cells; *m*, region of most active division; *p*, primary thallus; *r*, secondary rhizoids; *s*, region destined to become stem; *st*, stem; *t*, resorbed tip of primary thallus; *w*, region destined to become wing; *wi*, wing; ×13.

from the remainder of the thallus (fig. 22). This breakdown of the cells continues progressively from the tip toward the base of the

primary thallus. It usually begins at the same time as the formation of the lateral outgrowth, but it has been observed earlier. It continues until, by the time the stem and wing are differentiated, two-thirds or three-fourths of the primary thallus is broken down. The process is interpreted as being a phenomenon of resorption with the passage of the resorbed materials to the new growing region.

In place of the lateral outgrowth just discussed, a second thallus of the same type as the primary one may occasionally develop from the lateral meristematic region; such a thallus in its turn develops a slender stalklike portion and a broadened group of cells, at the base of which one or two lateral meristematic zones develop, just as on the primary thallus (fig. 23).

Under the cultural conditions discussed, the lateral outgrowth developed slowly, many of the plants died, and many others were destroyed during examination. The cultures also became overcrowded with algae, which were detrimental to the growth of *Riella*. It was further found that the narrow portion at the base of the primary thallus was easily broken off. It has been possible, to date, to grow only a single specimen to the stage of showing clearly the differentiation of stem and wing; this specimen was found 80 days after the cultures were sown (fig. 25).

The stem or axis appears on the proximal edge of the lateral outgrowth, that is, on the edge nearer the spore, and extends from the meristematic zone at the notch of the lateral outgrowth to the base of the primary thallus. This is accomplished by division of these marginal cells, which are already elongated, in the third plane to form an axis which is circular or oval in cross-section. This is the first time in the life history of *Riella* that cell divisions normally take place in the third plane. The falcate structure already described as extending from the growing point over the crest and back to the primary thallus constitutes the wing. The stem, therefore, is on the proximal margin of the lateral outgrowth (toward the spore), and the wing on the distal side (away from the spore). There is no sharp line of demarcation between the primary thallus and the new outgrowth, both of which lie in the same plane. The boundary line can be determined roughly by the fact that the cells at the base of

the new growth are somewhat larger than those of the adjacent primary thallus.

The plant at this stage is 2.6 mm. long from the base of the lateral outgrowth to the crest of the wing, and the maximum width of the wing is 0.44 mm. Since the margin of the wing has elongated more rapidly than the stem, it is a much folded and wavy structure, but shows no evidence of spiral twisting around the stem, as was at first indicated for *R. helicophylla*. The stem is 0.07 mm. wide at its distal curved region near the meristematic zone, and 0.18 mm. along most of its course, which is straight; along this straight portion it is five to seven cells wide. A portion of the primary thallus is still attached, and new rhizoids have developed from the base of the stem. There is no indication of sex organs, but trichomic outgrowths have made their appearance in the region of the growing point.

The early workers with *Riella* were much confused by the lateral outgrowth from the primary thallus and by the differentiation of the stem and wing, and their interpretations have differed materially. HOFMEISTER (8) calls attention to a lateral growing point, but he apparently thought of a growing region nearer the apex of the primary thallus; no apical cell is mentioned. He did not succeed in growing the sporelings to the point of stem and wing differentiation. TRABUT (15) does not discuss this stage, but his figures show what seems to be the primary thallus with a lateral outgrowth. GOEBEL (5) had no sporelings available. HOWE and UNDERWOOD (9) did not succeed in growing sporelings beyond the early primary thallus stage; they thought, apparently incorrectly, that the upper portion of the primary thallus becomes the wing and the lower portion the stem. They thought that the stem in *R. affinis* was being formed as a result of divisions in the third plane at the base of the primary thallus; their illustrations, however, show small, lateral, intercalary outgrowths comparable with those found by the writer for *R. americana*, although no interpretation was made of these. SOLMS (13) found two lateral outgrowths at the base of the primary thallus; one of these continues to grow while the other is suppressed. He found the growing region in the new growth, but thought that its entire lower portion becomes the stem. He maintained that an apical cell is formed later, but that, in spite of its position, it cannot be con-

sidered intercalary. His plants, however, did not actually reach the stem stage. PORSILD (12) found the lateral outgrowth from the primary thallus, and its thickening to form the stem on the side nearest the spore, but he found no apical cell at any stage of development, not even after the stem and wing had been clearly differentiated. He also reported the presence of oil cells on the primary thallus, but found less regularity in their shape than is reported in the present paper. He, too, found that the cells of the primary thallus die and empty their contents after differentiation of stem and wing. A sharp boundary line is reported to exist between the primary thallus and the wing. GOEBEL (6) found one or two growing regions localized at lateral intercalary points. He found no sharp line of demarcation between the primary thallus and the wing, hence he does not consider the lateral outgrowth (stem and wing) as a new structure, different from the primary thallus, as was thought by SOLMS and PORSILD. He found no definite wedge-shaped apical cell in the meristematic region at the tip of the stem.

Symmetry

The peculiar position of the wing with respect to the stem has been the subject of much discussion. A part of the dispute over the question of symmetry has been based on conclusions from meager information. It has been pointed out that many of the workers had no sporelings at their disposal, or else did not succeed in growing them to a sufficiently adult stage to show the origin of the stem and wing. Furthermore, the terms symmetry, dorsal and ventral, and distal and proximal, have not always been clearly defined.

In the filamentous stage, the *Riella* sporeling is evidently radially symmetrical, all sides of the short filament having the same appearance. The factor which determines the angle of the first longitudinal wall in the filament has not been determined; probably it will be found to be light or gravity.

The primary thallus stage of *R. americana* is certainly bilaterally symmetrical; that is, a plane extending through the middle of the thallus, perpendicular to it, and from its distal point to its proximal region, divides it into halves which image each other. This bilateral symmetry remains until the lateral outgrowth begins to develop on

one side; or, in the case of development of lateral outgrowths simultaneously on both sides of the primary thallus, bilateral symmetry is evident for a much longer time. There is no reason for considering the flattened, unistratose primary thallus dorsiventral, if by that term is meant that there is a difference between the two faces, for the exposed faces of the cells are alike on both sides of the thallus.

Other species of *Riella* exhibit the same condition in the primary thallus stage, as indicated by the descriptions and figures of HOFMEISTER (8), GOEBEL (5, 6), HOWE and UNDERWOOD (9), SOLMS (13), and PORSILD (12). A difference may exist in those species which grow horizontally on the substrate. Furthermore, GOEBEL (6, 7) drew a close parallel between the primary thallus of *Riella* and the gemma of *Marchantia* or *Lunularia:* in both of these cases there are one or two lateral intercalary growing regions from which the adult thalli arise; both gemma and primary thallus grow vertically at first; and the two flattened surfaces of both gemma and primary thallus are alike, the two faces of the gemma becoming different only after this structure has been in contact with the substrate for a time.

Confusion in the terminology of symmetry is mostly in evidence in connection with the lateral outgrowth, the young plant with differentiated stem and wing, and the adult plant. HOFMEISTER (8) compared the adult plant with the thallus of *Marchantia* in which one of the wings has been suppressed. LEITGEB (10) considers the wing a dorsal outgrowth of the stem, comparable with a comb standing at the tip of the stem; such a conception, however, implies that the stem develops before the wing, which is not the case. GOEBEL (5) recognizes some similarities to the thallus of *Marchantia*, but places emphasis on the fact that *Riella* grows in an erect position from the beginning (his "Profilstellung"). In a later work (7) he states that the apparently radial thallus is in reality an extraordinary modification of the usual dorsiventral thallus of the liverworts. Those species with a creeping, horizontal stem differ from the usual form of the liverworts only in that the wing is developed in a vertical position. The relationship between the wing and stem is similar in the species which grow in an erect position. He therefore considers the wing as dorsal to the stem. HOWE and UNDERWOOD consider the adult plant bilaterally symmetrical in the plane of the wing,

which is dorsal in relation to the stem. CAVERS (3) considers the wing dorsal and the stem ventral.

In the discussion of the lateral outgrowth and the adult plant of *R. americana* which follows, an effort is made to clarify the terminology of symmetry. The lateral outgrowth of the American species is from the beginning irregular, the cells nearest the spore (proximal) being smaller than those farther away (distal). The shape of the outgrowth is not perfectly semicircular, nor later absolutely circular; no plane at right angles to its surface will divide it into parts which image each other. The same is true for the older plant, in which stem and wing have been differentiated, and also for the mature plant. If the lateral outgrowth or the adult plant can be considered bilaterally symmetrical at all, it must be in the plane of the wing, as noted by HOWE and UNDERWOOD. Dorsiventrality, as just defined, is not exhibited at any stage in the development from the beginning of the lateral outgrowth to the maturity of the plant, for the two faces of the young lateral outgrowth, as well as of the stem and wing of the older plants, are alike.

As the lateral outgrowth develops, the angle which it forms with the primary thallus varies greatly; but as its development continues, a simple bending by differential growth on two opposite sides of the stem results in the adult plant being erect or ascending. In nature the mature *R. americana* is a much branched plant; only a few of the branches are definitely vertical, while most of them are ascending at various angles from 45° to 90° with the substrate. It is therefore logical to discard the terms dorsal and ventral, as used by most workers with *Riella*, who consider the wing dorsal and the stem ventral. A better terminology is to consider the wing distal with regard to the stem, using the position of the spore as a point of reference.

Summary

1. Spores of *Riella americana* were germinated in water and studied mainly in the living condition. There is little or no swelling of the spore, but oil drops are present in great numbers and a large vacuole forms at a position opposite the point of rupture.

2. The spore ruptures at the middle of the outer face, and a germ tube containing great numbers of oil drops emerges through the

spore wall, leaving a rounded opening. By apical growth a filamentous stage is produced, consisting of four to seven cells, which soon show numerous chloroplasts. The primary rhizoid originates from the germ tube between the spore and the nearest cross wall.

3. Beginning with the apical cell, most of the cells of the filament divide by longitudinal and cross divisions to form a broadened primary thallus one cell in thickness, the base of which remains as a meristematic zone. The secondary rhizoids develop as extensions of some of the cells between the spore and the broadened primary thallus. All rhizoids are smooth, colorless, and of uniform diameter.

4. The meristematic zone becomes localized at one or both sides, and one or two lateral outgrowths result which are one cell thick and in the same plane as the primary thallus, the tip of which is resorbed. The distal portion of the lateral outgrowth (away from the spore) becomes the wing of the adult plant, and the proximal margin (toward the spore) thickens by cell divisions in the third plane to form the stem. By a bending of the axis of growth the adult plant becomes erect or ascending.

5. The filamentous stage is radially symmetrical, and the primary thallus stage bilaterally symmetrical. The later stages, from the beginning of the lateral outgrowth to the adult plant, are not dorsiventral and are bilaterally symmetrical only in the plane of the wing. The wing is simply considered distal to the stem, using the position of the spore as a point of reference.

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REGENERATIVE CAPACITIES OF LEAF AND LEAFLET CUTTINGS OF TOMATO AND OF LEAF AND SHOOT CUTTINGS OF POTATO

C. L. ISBELL

(WITH TWENTY-THREE FIGURES)

Introduction

It is well known that the tomato plant is easily regenerated by stem cuttings, but available literature fails to reveal any work on the regenerative power of leaf and leaflet cuttings. Such an investigation was made by the writer during 1928 and 1929. Both leaf and leaflet cuttings readily regenerated roots, but only the former regenerated shoots.

The responses of leaf and leaflet cuttings of the tomato suggested the possibility that leaf cuttings of the Irish potato possessed similar powers of regeneration. KUPFER[1] reported that leaf cuttings of the Irish potato gave three distinct types of response: regeneration of roots; regeneration of a true tuber; and enlargement of the end of the petiole into a starch-bearing, tuber-like organ that might or might not regenerate roots. The results of experiments conducted in 1929 indicated that entire leaf cuttings, including no part of the stem, do not regenerate true tubers. This did not entirely agree with KUPFER'S interpretation of results with leaf cuttings. To check the results obtained in 1929 as against those of KUPFER, the experiments were continued in 1930. This paper reports the results of investigations with the tomato during 1928 and 1929, and with the Irish potato during 1929 and 1930.

Materials and methods

All material was shaded, protected from drying winds, and handled as rapidly as possible from the time it was collected until the cuttings were placed in the propagating bench. Sand was packed around the bases of the cuttings as is usually done with those of

[1] KUPFER, ELSIE, Studies in plant regeneration. Mem. Torr. Bot. Club **12**:195–241. 1907.

softwood. Specimens were removed from the propagating bench at frequent intervals and examined, care being taken to protect them from injury from drying. The bench was exposed to the sun except very late in the afternoon. A glass sash cover, used to maintain high humidity during the daylight hours, especially during the hottest part of the day, was removed at night.

Investigations

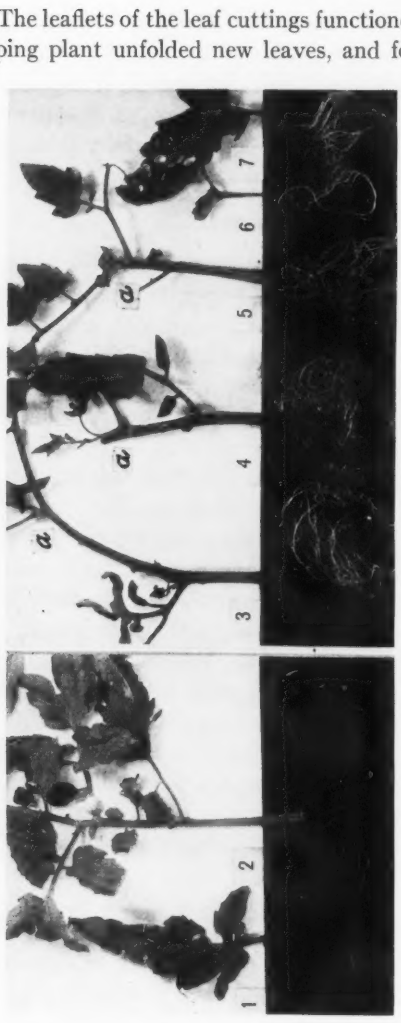
I. Lycopersicum esculentum

Cuttings consisting of entire leaves, carefully removed from the plant so that no part of the stem was included, were made in August, 1928. In May, 1929, additional cuttings of this type were made as well as others consisting of leaflets. After roots were produced, the plants were either potted and grown in the greenhouse or planted and grown in the garden.

Roots were readily regenerated by both leaf and leaflet cuttings, while only leaf cuttings regenerated shoots. Ten to fifteen days were required for the cuttings to become well rooted. Most of the roots appeared from the callus at the base of the petiolules and petioles, as shown in figs. 1 and 2 respectively. A few appeared on the petioles and petiolules above the callus, as shown in figs. 5 and 7 respectively.

Shoots began to appear on the leaf cuttings in the axils of the leaflets soon after the cuttings were potted or planted. The new shoots tended to develop toward the terminal parts of the leaf cuttings (fig. 3*a*), unless the cuttings were headed back, in which case they appeared in the more terminal axils remaining (figs. 4, 5). Each shoot developed a decidedly club-shaped enlargement toward its base, but there was a necklike constriction at the point of its attachment to the rachis. As a result of this constriction, the union of the shoot and rachis was usually not very strong (fig. 4). As the new shoot developed, the petiole and rachis enlarged and served as the main stem for the new plant (fig. 8*a*). The enlargement occurred rather slowly, as is typical of the basal part of the stems of stunted or hardened tomato plants. Some of the cuttings which had differentiated shoots in the axils of the leaflets later differentiated one or more shoots from the stem near the surface of the soil. Frequently these shoots became the most vigorous and formed the main part of

the plant. The leaflets of the leaf cuttings functioned as foliage until the developing plant unfolded new leaves, and for a considerable



FIGS. 1–7.—Fig. 1, leaflet cuttings; fig. 2, leaf cutting; photographed 24 days after cuttings were made; figs. 3–5, leaf cuttings: *a*, shoots arising in axils of leaflets; figs. 6, 7, leaflet cuttings photographed 37 days after cuttings were made.

time thereafter; but as the new plant increased in size and age the original leaflets gradually withered and died (fig. 8*b*), as do the more basal leaves of a normal tomato plant.

While leaflet cuttings formed roots as did leaf cuttings, they did not differentiate shoots (figs. 6, 7). Although kept under observation a month or more longer than the time required for leaf cuttings to differentiate shoots, it is possible that the leaflet cuttings were not observed long enough to determine definitely whether they

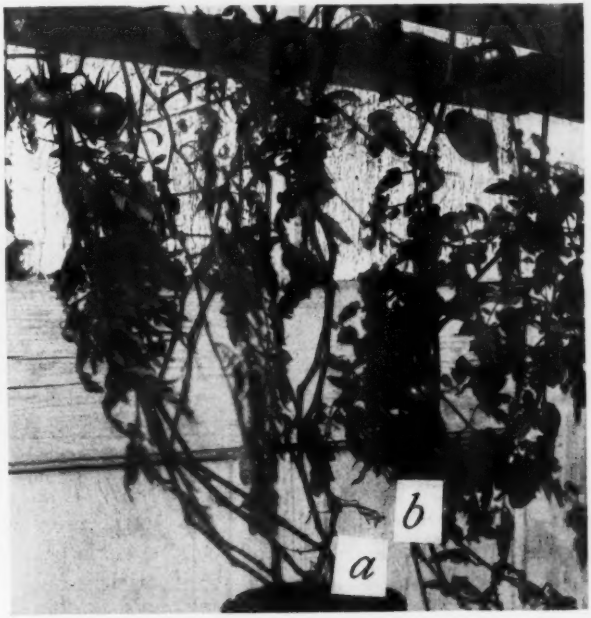


FIG. 8.—Plant from leaf cutting: *a*, petiole and rachis developed into main stem; *b*, original leaflets.

would differentiate shoots; the environmental conditions, moreover, may not have been favorable for shoot differentiation.

No special effort was made to obtain fruits from any of the plants regenerated by the leaf cuttings. Some of the cuttings taken in 1928, however, were kept in the greenhouse while others were planted in the garden. Flowers and fruits were produced by plants grown in both locations (fig. 8).

II. SOLANUM TUBEROSUM

EXPERIMENT I.—Early in the summer of 1929, an experiment was started with leaf cuttings of the Triumph variety. Within 3 weeks, despite injury to the foliage by a fungus, a number of the cuttings had formed roots and one had formed a tuber near the base of the petiole. The latter response suggested that since the tuber is an enlarged stem, there might have been left on the petiole of the cutting a rudimentary bud which developed into the tuber.

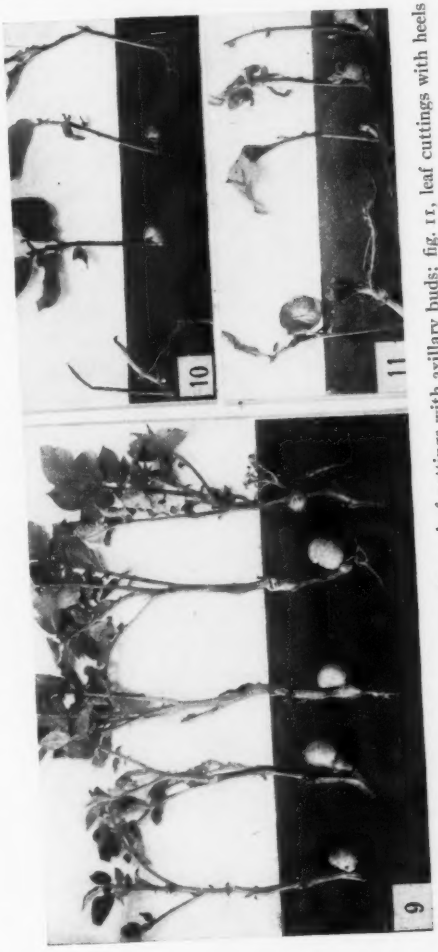
EXPERIMENT II.—To determine whether the tuber produced by one of the cuttings in experiment I was the enlargement of an axillary bud, three types of cuttings were taken July 1, 1929, from a late planting of the Irish Cobbler variety. These cuttings consisted of (1) the terminal 4–6 inches of stems with attached buds and leaves; (2) entire leaves with axillary buds carefully removed; and (3) entire leaves with a heel of the stem and axillary buds. These cuttings were handled as in experiment I and were similarly injured by a fungus. They were kept in the propagating bench until July 27, when they were examined and some of them photographed.

None of the cuttings without axillary buds developed tubers or shoots, whereas all of the others developed either a shoot, tuber, roots, or more than one of these in combination (figs. 9–11). The roots usually occurred on the new shoot, but a few stem and leaf cuttings with a heel of the stem regenerated roots at the base. The fact that cuttings with axillary buds readily regenerated tubers, while cuttings without axillary buds did not, strongly indicated that the tuber produced by one of the cuttings in experiment I developed from an axillary bud which had been overlooked and left on the base of the leaf petiole.

EXPERIMENT III.—To check the results obtained in experiments I and II, and the observations reported by KUPFER, another experiment was started April 14, 1930. Forty entire leaf cuttings with axillary buds, and 35 without axillary buds, were made from the Triumph variety. Leaves of different ages and from different locations on the plant were used in each case.

Ten days after the experiment was started 37 of the 40 cuttings with axillary buds had converted the axillary bud into one or more tubers (fig. 12). One had died from petiolar infection; the other two,

representing small young leaves, had not produced tubers. None of the cuttings without buds had produced tubers, but the basal part

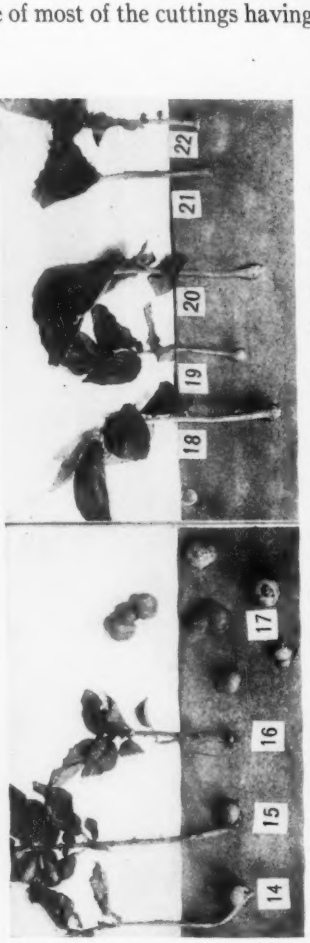


FIGS. 9–11.—Fig. 9, stem cuttings; fig. 10, leaf cuttings with axillary buds; fig. 11, leaf cuttings with heels of stem and axillary buds.

of the petiole of some of them was enlarging (fig. 13) and developing the pink color characteristic of the skin of the Triumph potato. Thirty-two days after the experiment was started, all with axillary

buds, except the one that died early, had developed one or more tubers (figs. 14–17). The foliage of most of the cuttings having died,

FIGS. 12, 13.—Fig. 12, leaf cuttings with axillary buds, photographed 10 days after cuttings were made; fig. 13, leaf cuttings without axillary buds, photographed 10 days after cuttings were made.



FIGS. 14–22.—Figs. 14, 15, potatoes developing from axillary buds and foliage dying; fig. 16, very young when cutting was taken; fig. 17, potatoes developed from older and larger leaves that died, photographed when 32 days old. Figs. 19, 20, 21, leaf cuttings without axillary buds; fig. 22, leaf cutting with axillary bud; side views, photographed when 32 days old.

the potatoes were harvested and weighed. They ranged from 0.105 to 5.805 gm., with an average weight of 2.61 gm. per plant. Some of the potatoes were allowed to cure and to go through a rest period

for 34 days, during which time they were exposed to light and developed chlorophyll. These were then planted in potting soil. Within 13 days after planting, some of the eyes were beginning to swell, and in 30 days the terminal bud had started growth. A photograph was made of one of these on August 3 (fig. 23). One of the potatoes produced from an axillary bud was overlooked and left in the propagating bench. It produced a shoot July 17, 193C.

FIG. 23.—Tuber produced from leaf cutting giving rise to normal plant.

The foliage of practically all of the cuttings without axillary buds was alive 32 days after the experiment was started. None of these cuttings had developed tubers, but the basal part of the petiole of several had enlarged considerably, the enlargement occurring (except in one instance) only opposite the axillary side (figs. 18–21). The cuttings were kept in the propagating bench and examined from time to time for 66 days, when all tops were dead. The enlarged bases were taken up and an attempt was made to let them go through a rest period preparatory to making a sprouting test, but all shriveled and died soon after they were removed from the soil.

Discussion

The axillary areas located at the bases of the more terminal leaflets of tomato leaf cuttings possess the power of terminal dominancy, and express it by regenerating shoots more quickly than similar but more basal axillary areas. The axillary areas of the most terminal leaflets remaining on headed-back leaf cuttings exhibit terminal dominancy by regenerating shoots more quickly than the more basal

axillary areas. These responses are interesting because of their similarity to the dominancy expressed in many plants by terminal buds and buds just below pruning cuts.

The behavior of the tomato leaf cuttings differed from the potato leaf cuttings in that the former readily regenerated roots, and also regenerated shoots in a comparatively short time, while true leaf cuttings of the latter regenerated few or no roots and failed to regenerate shoots. The regenerative responses of leaf cuttings of the potato not only differed greatly from the responses obtained with leaf cuttings of the tomato, but the former gave different responses, depending on whether the cutting carried an axillary bud.

There was a general tendency for the larger, more basal leaf cuttings to make larger tubers and to make them more quickly (figs. 12, 14–17).

The rapidity with which leaf cuttings with axillary buds converted the buds into tubers and the size of the tubers produced in a given time are relative measures of the importance of the leaf area to the potato plant. These also suggest that potato leaves with axillary buds might be used in a laboratory method to determine the extent to which injury to the foliage, caused by certain insects or diseases or other causes, affects the metabolic processes concerned in shoot, root, and tuber formation. It appears, moreover, that this information might be used in a practical way to show the grower how essential it is to protect foliage from injury of any kind, especially with quick maturing plants like the potato. The foliage also died in a shorter period on the older and larger leaf cuttings. This difference in behavior supports the belief held by some that the amount of leaf area and the age of the cutting influence the amount of food manufactured and the nature of the regenerations obtained.

The basal part of the older leaf cuttings without axillary buds appeared to enlarge earlier and die more quickly than was true with the younger leaf cuttings. This is shown by different cuttings in figs. 18–21. Fig. 21 is the oldest leaf; its foliage has practically died and dried. The photograph of the material shown in figs. 18–22 was taken 32 days after the cuttings were made.

Some of KUPFER'S drawings are similar to figs. 18–20, and show the enlargement of the base of the petiole to be opposite the axillary area. Her drawing of a leaf cutting that produced a tuber showed

the tuber on the axillary side of the petiole. This is identical with the behavior of leaf cuttings with axillary buds shown in figs. 12, 14–17, 22. On these regenerated tubers small leaves and eyes may be noted; KUPFER's illustration also shows rudimentary leaves and possibly eyes. The petioles which enlarged at the base and formed a potato-like skin failed to develop leaves or eyes, while the tubers from cuttings with axillary buds formed both. Potatoes produced from axillary buds went through a rest period, and had the power to develop green color in the presence of light and to grow when planted; whereas enlarged petioles without axillary buds died when taken up. Therefore, results of experiments reported in this paper strongly suggest that KUPFER misinterpreted some of her data on regeneration with Irish potato leaves. The tuber produced by a leaf cutting in her studies was presumably developed from an axillary bud which was unknowingly left on the cutting. It may be possible to produce tubers under some conditions, and with some varieties of leaf cuttings, without axillary buds, but observations made during the experiments herein reported are to the effect that, should this occur, the tubers would most likely develop on the petiole opposite the axillary side.

Summary

1. An investigation was made to determine the regenerative capacities of leaf and leaflet cuttings of the tomato, and of stem and leaf cuttings of the Irish potato.

2. Leaf cuttings of the tomato regenerated roots from the petioles, and shoots in the axils of the leaflets and from the petiole. Some specimens produced flowers and fruits. Leaflet cuttings regenerated roots but had not differentiated shoots when the experiment was discontinued.

3. Stem cuttings of the potato converted one of the axillary buds into a tuber, tuber and roots, or a tuber in combination with a shoot and roots. Some produced roots directly from the base of the cutting. Potato leaf cuttings with axillary buds converted the axillary bud into either a shoot or a tuber.

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NEW OR OTHERWISE NOTEWORTHY COMPOSITAE. VII

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 421

EARL EDWARD SHERFF

BIDENS KILIMANDSCHARICA **retrorsa** var. nov.—A specie achaeniorum aristis ad apicem saepissime retrorsum hamosis differt.

Specimens examined: *A. E. Haarer* 1472, at altitude of about 4000 feet, Doloti, Moshi District, German East Africa, August, 1928 (type in Herb. Kew).

BIDENS DIVERSA **megaglossa** var. nov.—A specie bracteis exterioribus ciliatis, ligulis 7–13 mm. longis differt.

Specimens examined: *Newton*, Biballa, Serra da Chella, Angola, Portuguese West Africa, June 3, 1883 (2 type sheets in Herb. Berl.).

Bidens mossii nom. nov.; *Coreopsis tripartita* M. B. Moss, Kew Bull. **1929**:184 and 196. 1929.—A species deceivingly like *Bidens whytei* Sherff, from which it appears to differ, however, in its larger and less dilated exterior involucral bracts, its exaristate achenes, etc.

BIDENS SETIGERA var. **abyssinica** (Schz. Bip.) comb. nov.; *Chrysanthellum* (subgenus *Microlecane*) *abyssinicum* Schz. Bip., Flora **25**:440. 1842; Walpers Repert. **6**:171. 1846; *Microlecane abyssinica* (Schz. Bip.) Benth. & Hook. *ex* O. Hoffm. *in* Engler und Prantl Pflanzenfam. 4^{v}:240, fig. 118*C*. 1894.

The achenes of this variety lack aristae but have the pappus more or less definitely coroniform or cup-shaped (whence the name *Microlecane* given by SCHULTZ BIPONTINUS). TEGETMEYER (O. Hoffm. *loc. cit.*) has illustrated this peculiar pappus in its extreme development.

In his own original treatment SCHULTZ BIPONTINUS referred this form, as representing a subgenus, to *Chrysanthellum*. The affinity with that genus seems, however, purely fallacious. BENTHAM and HOOKER (*loc. cit.*) elevated the subgenus, which SCHULTZ BIPONTINUS had named *Microlecane*, to the rank of a genus separate from *Chrysanthellum*. However, through *Bidens setigera* Sherff and *B.*

setigeroides Sherff the type material of *Microlecane* is too closely allied with *Bidens* to justify generic segregation. Thus, for example, *B. setigera* has very long, slender aristae and many of the achenes are much the same as in other species of *Bidens;* but a fair percentage of them have the shorter or secondary pappus noticeably modified into an annular or cupuliform corona, thus suggesting the so-called *Microlecane* material, here treated as the var. *abyssinica*. All things considered, it seems that the var. *abyssinica* bears somewhat (although not entirely) the same relationship to *B. setigera* proper that *Bidens aristosa* var. *mutica* (Gray) Gatting. bears to the species *B. aristosa* (Michx.) Britt. proper.

BIDENS TRIPLINERVIA var. MACRANTHA **octoradiata** f. nov.—E var. *macrantha* capitulis regulariter 8-radiatis differt.

Specimens examined: *R. Hauthal* 273, alt. 3600–4800 m., vicinity of La Paz, Bolivia, January, 1906 (Herb. Berl.); *Pflanz* 406, alt. 3550 m., schist slope, Chullo, Palca, La Paz, Bolivia, Mar. 13, 1910 (type in Herb. Berl.); *A. Mathews* 571, Peru, April (Gray); *Eduard Seler*, Sierra Chica, Province of Córdoba, Argentina, Apr. 1, 1910 (Herb. Gray); *idem et Mrs. Caecilie Seler* 3021, alt. 3000 m., Cordillera between Todos los Santos and Chiantla, Guatemala, Sept. 11, 1896 (Herb. Berl.); *A. Stübel* 43*b*, Peru, April-June, 1875 (Herb. Berl.); *Dr. A. Weberbauer* 435, alt. 3700 m., Peru, Feb. 24–25, 1902 (Herb. Berl.).

Bidens triplinervia H.B.K. and its varieties are ordinarily separable from *B. andicola* H.B.K. and its varieties by means of the number of rays to each head. In the former these number usually five, in the latter usually eight. In the case of *B. triplinervia* var. *macrantha*, however, there occur very rarely forms in which all the heads are eight-rayed.

BIDENS GRACILIOR **ukerewensis** var. nov.—Folia bi-tripinnatipartita, segmentis ultimis linearibus moderate adpresso-hispidis plerumque 1–1.5 mm. latis; achaeniis exterioribus corpore circ. 4.5–5 mm. longis interioribus 6–7 mm. longis, omnibus tenuiter biaristatis aristis 0.5–1 mm. longis apice retrorsum 1–4-hamosis.

Specimens examined: *Dr. Carl Uhlig* 19, abundant along the shoreline of Lake Victoria Nyanza (L. Ukerewe), Ukerewe, German East Africa, Apr. 20, 1904 (type in Herb. Berl.).

BIDENS SUBALTERNANS DC. Prodr. **5**:600. 1836; *B. platensis* Mang., An. Mus. Nac. Buenos Aires **24**:230. 1913.—*Bidens platen-*

sis Mang. was described as a hybrid between *B. pilosa* L. (staminate) and "*B. bipinnata* L" (pistillate). A study of MISS MANGANARO'S text some time ago convinced me that the plant which had been assumed to be *B. bipinnata* L. was in reality *B. subalternans* var. *simulans* Sherff. Recently I was given valuable aid in the determination of *B. platensis* itself by PROFESSOR ANGEL L. CABRERA of the La Plata Museum in Argentina. In a letter he wrote to the following effect: His numerous searches in the past for the type of *B. platensis* had been fruitless. He therefore had given the type up for lost. Recently, however, he had been entrusted with the rearrangement of the SPEGAZZINI collections for the La Plata Museum. Now, the late MISS MANGANARO had been a pupil of SPEGAZZINI, and at her death her parents sent her collections to SPEGAZZINI. PROFESSOR CABRERA found her collections inside an old chest, in November, 1930. The herbarium was in great disorder, without labels or any other indications. However, there were several specimens of *Bidens* that had been very carefully mounted, and these were "closely similar to the original figure of *B. platensis*." Since no other specimens in the Manganaro collection had been given such special care, PROFESSOR CABRERA concluded (doubtless rightly) that these specially mounted ones represented the type material of *B. platensis*. He generously lent me two of the supposedly type specimens for study and they are now before me. Both are typical *B. subalternans* DC.

Bidens phelloptera sp. nov.—Herba verisimiliter perennis, usque ad 2 m. alta, caule obscure angulato glabro. Folia secundaria (primaria non visa) tenuiter petiolata petiolis ∓1.5 cm. longis petiolo adjecto ∓4.5 cm. longa, pinnatim 3–5-partita, segmentis valde membranaceis ovatis vel lanceolatis, apice acutis, supra glabratis subtus pubescentibus, circ. 6–13 mm. latis, acerrime dentatis dentibus mucronatis. Capitula corymbose disposita, ramulos plerumque glabros usque ad 8.5 cm. longos terminantia, radiata, pansa ad anthesin ∓4 cm. lata et circ. 10–11 mm. alta. Involucri glabri bracteae exteriores 8–10, lanceolatae vel lineari-oblongae, apice obtusae, mox valde reflexae; interiores late oblongo-lanceolatae, basaliter vel etiam usque ad medium connatae, demum circ. 8.5–9.5 mm. longae. Paleae late lineari-oblongae, apicaliter obtusae atque atro-coloratae, 7–9.5

mm. longae. Flores ligulati ∓6 (normaliter 8?), flavi, ligula anguste obovati, apice minute acriterque 3–4-denticulati, 1.7–2 cm. longi. Achaenia valde obcompressa, unaquaque duarum facierum subatra 8-sulcata glabra vel apicem versus erecte setosa, marginibus crassiusculis densissime erecto-ciliata, exteriora late oblonga 5–6 mm. longa et marginibus perspicuis incrassatis inclusis 2–2.4 mm. lata apicaliter calva vel breviter biaristata et saepe paucisetosa, interiora lineari-oblonga corpore circ. 8–9 mm. longa et marginibus angustis inclusis circ. 1.5–1.8 mm. lata, apice plus minusve setoso biaristata aristis tenuibus usque ad 2.5 mm. longis, nunc glabris nunc antrorsum vel etiam retrorsum 1–2-hamosis.

Specimens examined: *Dr. W. Busse* 2257, alt. about 750 m., south slope of Mt. Gonja, Usambara, German East Africa, Apr. 18, 1903 (type in Herb. Berl.).

The branches of the type material have internodes 1–1.5 dm. long. *Busse's* label lists the plant as an herb and gives its height as being up to 2 meters. The interior involucral bracts are connate below, often even up to the middle, thus simulating those of the allied genus *Thelesperma*. The general habit, however, in no way suggests *Thelesperma*. The achenes are thick-margined and suggest those of some species of *Coreopsis*, but the occasional retrorse awn barbs are foreign to that genus.

Bidens personans Degener & Sherff sp. nov.—Perennis, supra herbacea, erecta, glabra, verisimiliter 3–7 dm. alta, caule ramisque valde tetragonis. Folia tenuiter petiolata petiolis sparsim ciliatis (1–) 2–5 cm. longis, petiolo adjecto 7–12 cm. longa, principalia tripartita, foliolis ovato-lanceolatis vel suboblongo-lanceolatis, membranaceis, glabris et eciliatis, serratis dentibus indurato-apiculatis, lateralibus sessilibus vel parce subsessilibus, omnibus apice acutis vel terminali breviter acuminato. Capitula corymboso-paniculata, numerosa, pedicellata pedicellis tenuibus hispidisque, radiata, pansa ad anthesin circ. 1.3–1.7 cm. lata et circ. 4 mm. alta. Involucri inferne plus minusve hispidi bracteae exteriores circ. 4–6, lineari-oblongi, patentes, saepius ciliatae, apice obtusae, tantum circ. 1.5–2 (raro −2.5) mm. longae, quam interiores lanceolato-oblongae apice minute pubescentes dimidio breviores. Flores ligulati plerumque 5, flavi, ligula late oblanceolati, apice minute denticu-

lati, circ. 7–9 mm. longi. Achaenia anguste linearia, infra medium recta supra (saltem apicem versus) valde curvata, glaberrima, nitide brunneo-nigra, exaristata, valde obcompressa, non manifeste striata, exalata, circ. 1–1.2 cm. longa et ∓0.7 mm. lata, demum paleas oblongo-lineares valde superantia.

Specimens examined: *William Bush* 32, alt. 1200 ft., on south slopes in semi-arid valley, Palikea, Isl. Oahu, Hawaiian Isls., Jul. 7, 1929 (type, Herb. Field Mus., 2 sheets: cotypes, Herb. Berl.; Herb. Kew).

Offering deceptive resemblances (whence the trivial name) to *Bidens fulvescens* Sherff in texture and color of foliage; to *B. sandvicensis* Less. and *B. conjuncta* Sherff in the herbaceous, acutely tetragonal branches and upper portions of stem, and to *B. conjuncta* further in the sessile lateral leaflets.

Bidens oligocarpa sp. nov.—Herba annua, erecta, gracilis, 4–6 dm. alta, caule ramisque acriter tetragonis et sparsissime hispidis. Folia petiolata petiolis tenuibus ciliatis usque ad 1.5 cm. longis, petiolo adjecto 4–8 cm. longa, pinnatim 3–5-partita, foliolis ovatis vel oblongo-lanceolatis, valde membranaceis, acriter serratis dentibus indurato-apiculatis, faciebus sparsim minuteque adpresso-hispidis, marginibus ciliatis, terminali usque ad 2 cm. lato et apice acuminato. Capitula pauca, ramos (pedunculos) tenuissimos usque ad 8 cm. longos terminantia, radiata, pansa ad anthesin circ. 1.2–1.6 cm. lata et 5–7 mm. alta. Involucri subglabri bracteae exteriores 5–7, tenuissime lineares, hispido-ciliatae, supra moderate dilatatae, apice acerrimae, 1.5–2.3 mm. longae; interiores late lanceolatae vel oblongo-ovatae, apice pubescentes, 3–3.5 mm. longae. Flores ligulati 4–5, rosacei, ligula oblongi vel anguste obovati, apice obtuso obsolete denticulati, 7–9 mm. longi. Achaenia circ. 6–8, linearia, obcompresso-tetragona, omnino circ. 8– (unica facie 2–) sulcata, atra, superne sensim angustata, exalata, plus minusve erecto-hispida setis e faciebus ipsis vel e papillis ortis, corpore 6–10.5 mm. longa et 0.7–0.9 mm. lata, apice biaristata aristis retrorsum hamosis, 0.4–1 mm. longis.

Specimens examined: *C. E. Lloyd* 409, State of Sonora, Mexico, 1890 (type in Herb. Gray).

BIDENS SKOTTSBERGII var. **conglutinata** (Deg. & Sherff) comb. nov.; *B. hawaiensis* var. *conglutinata* Deg. & Sherff, BOT. GAZ.

89:364. 1930.—At the time the var. *conglutinata* was described, *B. skottsbergii* had not been published. A study of the achenial characters now shows that the variety has the achenes of *B. skottsbergii*, and in fact matches that species rather well except that the species has noticeably larger rays.

Ericentrodea decomposita Blake & Sherff sp. nov.—Scandens, verisimiliter fruticosa, caule obtuse angulato moderatim hispido pilis multiloculatis, divaricate ramoso. Folia opposita, principalia divaricata, petiolata petiolis marginaliter dense ciliatis aliter sparsim pilosis (omnibus pilis multiloculatis) 5–7 cm. longis, petiolo adjecto ±1.5 dm. longa, plerumque quadriternata, segmentis indivisis vel rursus lobatis, segmentis vel lobis ultimis diverse oblongis vel rhomboideis vel lanceolato-ovatis plerumque 1–3-dentatis dentibus apicaliter rotundatis et abrupte mucronulatis, marginibus sparsim ciliatis pilis saepe multiloculatis, facie superiore glabra coriaceaque, facie inferiore glabrata vel ad costas (praecipue medianam) moderate pilosis pilis elongatis multiloculatisque. Capitula cymoso-paniculata, tenuiter pedicellata pedicellis hispidis circ. 1–2 cm. longis, discoidea, pansa ad anthesin circ. 11–13 mm. lata et circ. 8–9 mm. alta, (ex floribus tubulosis siccis) flava. Involucri hispidi bracteae exteriores 8–10, tenuiter lineares vel subulatae, apicaliter acerrimae, 3.5–4.5 mm. longae, interiores lineari-lanceolatas paulo superantes. Paleae oblongo-ovatae, subbrunneae, marginaliter diaphanae, demum circ. 4.5–5 mm. longae. Flores tubulosi ±35, subcylindrici, infra non manifeste in tubum contracti, hermaphroditi, corolla circ. 5 mm. longi, limbo 5-dentati; stylorum ramis apice triangulatis, acuminatis, papillosis. Achaenia (submatura!) plana, oblanceolata, utrinque truncata, faciebus brunneo-nigris obscure lineata et glabra vel apicem versus raro erecto-setosa, marginibus densissime pectinato-ciliata pilis subbrunneis adscendentibus et longitudine corporis diametrum etiam superantibus, corpore tantum circ. 1.5–2 mm. longa et circ. 0.5 mm. lata, apicem versus non cervicata sed ad apicis ipsius latus quodque plerumque 3–4-aristata (itaque omnino plerumque 6–8-aristata) aristis basaliter rarius connatis, tenuibus, stramineis, 1.5–2 mm. longis, retrorsum hispidis barbatisve.

Specimens examined: *Dr. A. Weberbauer* 7075, Peru, 1909–1914 (type in Herb. Field Mus.).

In the key to the species of *Ericentrodea* presented some years ago by DR. S. F. BLAKE and myself (Jour. Wash. Acad. Sc. 13:104. 1923), three species were listed: *E. corazonensis* (Hieron.) Blake & Sherff, *E. homogama* (Hieron.) Blake & Sherff, and *E. mirabilis* (Sherff) Blake & Sherff. From *E. corazonensis* the present plant differs at once in having discoid, not radiate, heads and the lower or principal leaves mostly at least quadriternate, not just ternate. From *E. homogama* it differs in having the lower or principal leaves mostly at least quadriternate, not biternate, and in having much narrower ultimate segments to the leaves. From *E. mirabilis* it differs again in its mostly at least quadriternate, not biternate, lower or principal leaves; also in having ∓35-flowered heads about 11–13 mm. wide and about 8–9 mm. tall, not ∓12-flowered heads 3–5 mm. wide and about 4–5 mm. tall; in having exterior involucral bracts narrowly linear (or subulate) and smooth-edged (except for hairs), not linear-lanceolate and not laciniate-ciliate or serrulate; in having the achenial awns definitely arranged in two widely separated groups (one group over each lateral margin), instead of being subregularly awned with awns peripherally and equidistantly arranged at the achenes' apex; etc.

XANTHIUM PENNSYLVANICUM **laciniatum** Sherff & Shull, var. nov.—Some time before his death the late Mr. F. F. CREVECOEUR sent to PROFESSOR CHARLES A. SHULL of the University of Chicago some anomalous specimens of *Xanthium*. They had been found growing in a clump on loam soil at one end of a corn field belonging to MR. CREVECOEUR, near Onaga, Kansas, July 19, 1928. MR. CREVECOEUR wrote, "I have been told by several that they have seen the same form southwest of Onaga, but I have seen only those grown on my farm." Interested in the plants because of their laciniate foliage, he planted burs "in a highly manured garden and no plants exceeded 24 inches in height as guessed at. At any rate, none were 30 inches high." Burs from the CREVECOEUR material were planted by DR. SHULL in the summer of 1930, in the experimental garden of the University of Chicago. There I was very kindly permitted by him to make frequent observations and later to collect herbarium specimens for study and description.

The twenty or more plants raised under our observation were

strikingly uniform[1] in being somewhat dwarfed (only about 4–6 dm. high) and in having the leaves with rather small, deltoid blades (mostly 6–8 cm. wide), these laciniately 3–5-lobed, the sinuses sometimes extending even to the rhachis and the several teeth of each lobe becoming attenuate-linear. The fruits were exactly like those found on normal *Xanthium pennsylvanicum* Wallr.

Numerous specimens were collected and these will be distributed to important herbaria. The technical description follows: Plantae subpumilae, 4–6 dm. altae. Folia principalia petiolata petiolis tenuissimis plerumque 5–9 cm. longis, laminis deltoideis 6–8 cm. latis laciniatim 3–5-lobatis sinibus saepe profundis vel etiam usque ad rhachidem incurrentibus dentibus marginalibus sursum attenuato-linearibus.

Specimens examined: *Earl E. Sherff* 5012, cultivated in experimental garden of University of Chicago, Chicago, Illinois, Oct. 11, 1930 (type in Herb. Field Mus.).

CHICAGO NORMAL COLLEGE
CHICAGO, ILL.

[1] One plant in the culture was typical *X. pennsylvanicum* in all respects, but I assume that this had resulted from an admixture of burs from other stocks that had been used in making certain comparisons.

EMBRYOLOGY OF OSMUNDA CINNAMOMEA

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 422

GEORGE L. CROSS

(WITH EIGHT FIGURES)

Introduction

The external appearance, anatomy, and meristems of the Osmundaceae have been shown to represent a condition intermediate between the eusporangiate and leptosporangiate ferns, which led to the suspicion that the embryology might likewise show intermediate characteristics. Data of this kind should be of value in studies of classification and phylogeny. The uniformity of structure of the embryo of the more recent ferns is entirely lacking in those which are primitive. In some of the latter a suspensor is present, a feature not seen in recent ferns, although common in lycopods and seed plants.

The desirability of classifying ferns as eusporangiate and leptosporangiate is probably more apparent than real, but for want of something better such a classification still persists in the literature. To distinguish between the two groups, technicalities beyond the purpose of this paper are involved. BOWER states that eusporangiate ferns usually have more than a single apical cell involved in their meristems, that is, they usually have a group of master cells involved in growth at the stem tip, root tip, and in the production of sporangia. In the case of the leptosporangiate forms, practically all structures can be traced in origin to the activities of a single apical cell, with definite segmentation. There are also certain embryological differences which will be considered more in detail.

Although not all the families of the Filicales have been investigated embryologically, the embryo of a typical eusporangiate fern, such as *Marattia*, has been described by CAMPBELL (3) as follows:

> The first division of the embryo is perpendicular to the archegonium and parallel to the surface of the gametophyte. It divides the embryo into epibasal and hypobasal halves. Further divisions result in a quadrant stage, an octant stage, and finally a somewhat unsymmetrical mass of tissue in which the ap-

pendages are not clearly distinguishable as to their origin. Ultimately leaf, stem, and root are produced from the epibasal half of the embryo, while the entire hypobasal half is given over to the production of a massive foot.

In some groups a suspensor is produced, as described by LAND (7) for *Angiopteris evecta*. It is formed next to the neck of the archegonium, and by its enlargement pushes the embryo deep into the nutritive tissue of the gametophyte. The sporeling grows directly upward through the gametophyte.

The embryos of representative leptosporangiate ferns have been described by GOEBEL (5), LEITGEB (8), ATKINSON (1), SHAW (10), RAUWENHOFF (9), JANCZEWSKI (6), and others. The ordinary method of development is somewhat as follows: the first, or basal wall is laid down parallel to the longitudinal axis of the archegonium and perpendicularly to the anterior-posterior axis of the gametophyte. It divides the embryo into posterior and anterior halves. The second wall is laid down perpendicularly to the archegonium, but parallel to the surface of the gametophyte, producing the quadrant stage. It is at this stage that the position of leaf, stem, root, and foot are presumably determined. Each cell of the quadrant divides in such a manner as to outline definitely the apical cell of the leaf in the lower anterior quadrant; the stem in the upper anterior quadrant; the root in the lower posterior quadrant; and the foot in the upper posterior quadrant. Divisions in the foot proceed irregularly, however, and the wedge-shaped cell soon loses its individuality. As a result of the position of these primary organs, the young sporeling grows along under the gametophyte and usually comes up through the notch at the apex of the gametophyte.

Since the Osmundaceae have been placed taxonomically with the Leptosporangiates, it would be reasonable to suspect that the embryology would be similar to that of the typical leptosporangiate fern. The earliest and most frequently quoted work on the Osmundaceae was done by CAMPBELL (4) in 1892. The results of his findings with respect to the embryo are substantially as follows: The first wall of the embryo is laid down in a plane parallel with the main axis of the archegonium, presumably perpendicular to the anterior-posterior axis of the gametophyte, although this point is not made clear. The second wall is laid down parallel with the archegonium, and also parallel with the surface of the gametophyte. The embryo at this

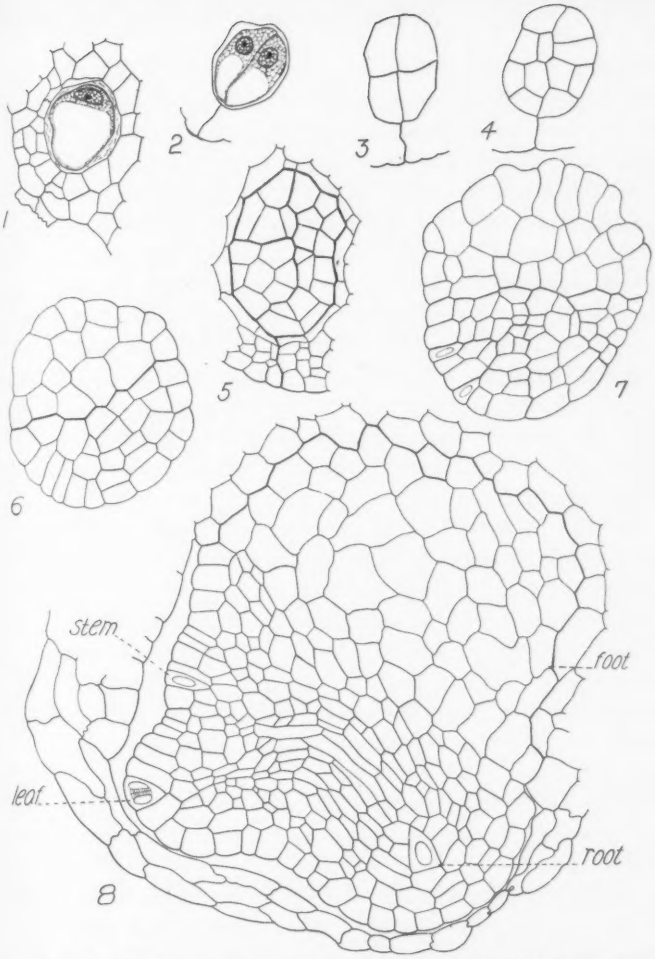
stage is composed of two anterior and two posterior quadrants. The third cell generation results in the production of the apical cell of the leaf in the lower anterior quadrant; the stem in the upper anterior quadrant; the root in the lower posterior quadrant; and the foot in the upper posterior quadrant. The foot becomes massive in the course of its development, a feature which suggests relationship with the Eusporangiates. The embryo usually grows out toward the side of the gametophyte, transverse to the main axis rather than parallel with it, although CAMPBELL does not make any special point of this fact. Obviously, according to CAMPBELL, the method of development is essentially that of a leptosporangiate fern, the main differences being the placement of the second wall parallel with the archegonium and the more massive foot.

Methods

Prothallia of *Osmunda cinnamomea* grown in the greenhouse and in the laboratory produced antheridia as early as the 6- or 8-celled stage, but owing probably to nutritive relationships, the production of archegonia was delayed until the prothallia measured 3–5 mm. in width. Various methods of killing and fixing were employed, but the best results were obtained with chromo-acetic acid in strengths of 0.5–1 per cent of each. Since the young sporophytes grow out at right angles to the gametophyte, sections were cut perpendicular to the anterior-posterior axis of the latter. Sections 8–10 μ were found to be most satisfactory.

Results

Fertilization in *Osmunda cinnamomea* occurs in the usual manner. In all the cases examined the first wall of the zygote was laid down parallel to the longitudinal axis of the archegonium, and parallel to, or at a variable angle with, the anterior-posterior axis of the gametophyte (fig. 2), instead of perpendicular to such axis as indicated by CAMPBELL (4). The second wall apparently may be placed either parallel or perpendicular to the archegonium (fig. 3). CAMPBELL states that it is at this stage that the point of origin of the primary organs is determined, that is, the stem and leaf from the anterior quadrants, and the foot and root from the posterior ones. It is not made clear, however, just how a quadrant stage arises from the placement of two walls, both parallel to the longitudinal axis of the archegonium.



FIGS. 1–8.*—Fig. 1, fertilized megagamete which has elongated and become vacuolate. Fig. 2, first wall of embryo; section parallel to archegonium and perpendicular to main axis of gametophyte. Fig. 3, quadrant stage, parallel to archegonium and perpendicular to main axis of gametophyte. Fig. 4, embryo just past octant stage, showing rectangular cells not related to origin of apical cells. Fig. 5, young embryo. Fig. 6, embryo showing origin of foot in upper half. Fig. 7, embryo with foot clearly delineated above and possible origin of leaf and stem in lower left portion; section parallel to archegonium and perpendicular to gametophyte. Fig. 8, embryo showing foot, leaf, stem, and root; section parallel to archegonium and perpendicular to gametophyte.

* All figures drawn at level of table with Spencer camera lucida, under 4 mm. Leitz apochromatic objective, with periplan ocular 8×; reduced to one-fourth original size.

To obtain a median section of such a quadrant, one would cut sections of the embryo in a plane perpendicular to the archegonium, in which case it would seem also that the appendages would arise in a plane perpendicular to the archegonium. It would preclude the possibility of obtaining longitudinal sections of the embryo in any but sections perpendicular to the archegonium, a concept not in accord with CAMPBELL's figs. 95 and 96, which show longitudinal sections of embryos in such a manner that a longitudinal view of the neck of the archegonium is also presented.

I was able to obtain the best longitudinal sections of embryos by orienting the thallus with its longitudinal axis perpendicular to the knife. This method showed that the quadrants had no fixed orientation with respect to the thallus, and, as will be shown later, specific quadrants are not correlated with the ultimate position of the primary organs.

Sometimes there is delay in formation of the walls of the embryo, and the embryo elongates along the major axis of the archegonium in such a manner as to suggest a suspensor (fig. 2). In such cases the nucleus migrates to the proximal end of the embryo, and the distal end becomes highly vacuolated. The vacuolated portion may be regarded as a rudimentary suspensor, although the situation occurs infrequently, and studies were correspondingly limited. If two megagametes on the same thallus are fertilized, the younger of the resulting embryos frequently passes through such stages, probably owing to scarcity of food. This tendency toward suspensor formation, if it may be regarded as such, is certainly suggestive of the eusporangiate ferns.

The quadrant stage is succeeded by the octant stage through the medium of a third generation of cell walls, four in number, which divide each of the quadrants into two octants, one of the latter being triangular in profile, the other rectangular, as described by CAMPBELL (4). The triangular octants resemble apical cells, but the same situation prevails in *Riccia*, *Marchantia*, and other forms with spherical embryos; and as such views may be obtained by cutting through the center of the embyro in two different planes, it does not follow that they are apical cells. In fact, subsequent divisions rapidly obscure the individuality of these cells, and a relatively large globular mass of tissue is produced, in which the foot is the only appendage

clearly discernible (figs. 6, 7). As may be seen by reference to these figures, the foot is distinct as such at a relatively early period in ontogeny, the line of demarcation between it and the rest of the embryo apparently being the second wall laid down in the zygote. Thus it seems that the foot arises from the entire half of the embryo next to the gametophyte, instead of from the upper posterior quadrant as described by CAMPBELL (4); although the origin of appendages may not be infallibly ascribed to definite halves, quadrants, or octants of the embryo. Probably a better concept of the situation is that the foot arises roughly from that half of the globular mass of tissue next to the gametophyte; and leaf, stem, and root arise from the half of the embryo abutting the neck of the archegonium (fig. 8).

The cells of the foot undergo enlargement, and to a limited extent division. The region thus keeps pace with the more actively dividing mass of cells to its exterior, with the result that the embryo retains, to some extent, its spherical shape, even though a relatively large mass of tissue is produced (fig. 7). This feature, of course, is much more suggestive of the eusporangiate than of the leptosporangiate ferns. The decline of cell divisions in the foot may be correlated with an extensive conduction of nutrient materials to the meristematic tissues beyond, a condition which in turn probably interferes with normal nuclear phenomena, and is facilitated by the large size of the cells involved. The rudimentary conductive system thus arising is considered by LAND to be the primary root of the embryo.

Fig. 7 represents what may be the origin of the leaf and stem apices from the upper distal quadrant, the root from the lower distal quadrant, and the foot from the proximal half of the embryo; but many sections in corroboration of this interpretation were not obtained. Some forty embryos were sectioned, and a preponderance of evidence favored an indeterminate origin of all appendages. This is in correlation with the situation found in the eusporangiate ferns. The suggestion of a suspensor, either extant in the past or to manifest itself in the future, still further suggests the latter group.

The epidermal and hypodermal layers of cells about the portion of the embryo next the calyptra, especially the region which is destined to give rise to the root, become thick walled and filled with tannins and oils. Interruptions in this protective thickened layer occur only at the stem and leaf apices, where mucilage is secreted to

prevent desiccation after the young plant has ruptured the calyptra. The tannin and oil laden cells, adjacent to the apical cell of the young root, act first as a protection to the root in its efforts to break the calyptra, and later become part of the root cap.

The apical cell of the stem arises from an epidermal cell of the embryo (fig. 8). It is usually about three times as long as it is broad, tetrahedral in shape, and of irregular segmentation.

The leaf initial arises usually very close to the stem tip, possibly from the same quadrant as suggested above (fig. 7). It is a triangular pyramidal apical cell with regular segmentation, in contrast to the condition found in most leptosporangate forms, where a 2-sided apical cell is the rule. It is always oriented with one of the flat sides toward the stem tip, a situation reported by BOWER (2) for leaves that are formed later. The segments derived from the two sides of the apical cell away from the stem tip divide more rapidly than the ones from the third, so the leaf is circinate.

The root initial arises endogenously in the same half of the embryo as the leaf and stem initials, instead of exogenously in the region described by CAMPBELL (4). It is first noticeable as a rectangular cell, difficult to distinguish from its neighbors until it has cut off one or two segments (fig. 8). It is usually somewhat enlarged, with transparent contents. It may take the form of a triangular pyramidal apical cell with four cutting faces, the usual case, or it may be a 4-sided pyramid with five cutting faces. The inner half of each lateral derivative of the apical cell contributes to the vascular system of the root, the outer half to the cortex. Shortly before rupture of the calyptra, the vascular system of the young embryo is outlined, continuously from the root tip to the first leaf, with the suggestion of a divergence of a portion of the strand to the second leaf (fig. 8).

According to CAMPBELL, the first leaf ruptures the calyptra by its growth. Studies of sections such as are represented by fig. 8 indicate that the calyptra, which has been distended and flattened by the growth of the embryo as a whole, is ultimately ruptured by the first soil root. The latter grows rapidly and penetrates the substratum before the first leaf reaches any appreciable size.

Summary

1. The first wall in the embryo of *Osmunda* is laid down in a plane parallel with the longitudinal axis of the archegonium, usually paral-

lel with the longitudinal axis of the thallus. The second wall is laid down parallel or perpendicular to the archegonium, forming a typical quadrant stage. An octant stage follows.

2. The origin of the primary appendages cannot be referred to definite halves, quadrants, or octants of the embryo.

3. The primary leaf, stem, and root are produced from approximately one-half of the embryo adjacent to the neck of the archegonium. The foot consists of the half of the embryo next the gametophyte.

4. The root is endogenous in origin.

5. The preceding features, when considered collectively, indicate that *Osmunda* stands nearer the eusporangiate than the leptosporangiate level when embryological evidence is considered. The two main points in common with the eusporangiate ferns are: (1) the origin of the leaf, stem, and root from the same half of the embryo, and the foot from the other half; (2) the occasional presence of an incipient suspensor. The contrasting points are: (1) the placement of the first wall with respect to the axis of the archegonium; (2) the direction of growth of the embryo. It seems logical to assume that the Osmundaceae may be intermediate between the eusporangiate and the leptosporangiate ferns with respect to embryological features.

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DEVELOPMENT OF SEX ORGANS OF FERN PROTHALLIA UNDER PROLONGED CULTIVATION

DAVID M. MOTTIER

(WITH TWO FIGURES)

Certain vigorous fern prothallia were transplanted to culture dishes upon rich soil and given plenty of space, and have been kept successfully in continuous growth for eight years. The plants themselves, or their continuation shoots (clones), have attained the size, and in general outline the appearance, of the liverwort *Marchantia* (fig. 1). Four years after the experiment was begun the results were published in this journal (**3**, **4**). The cultures have now been grown uninterruptedly for eight years, and thus the continuation shoots have been kept under constant observation. It would be possible to continue the cultivation indefinitely, if the difficulties encountered could be overcome.

In former publications the method of cultivation was described somewhat in detail, the difficulties which arose from time to time were mentioned, and remedies given. The most dangerous and annoying feature was the presence of the larvae of a small gnatlike dipterous insect (**4**). Fumigation of the greenhouse gave only temporary relief, for the gnats doubtless came from outside sources. They and their maggots appeared again in a comparatively short time, and it seemed that the cultures were doomed. Not only that, but new cultures started from spores were at times completely destroyed. In due time, however, and before the continuation cultures were seriously injured, an effective remedy was found which consisted in fumigating the cultures in which the larvae were found with the vapor of carbon bisulphide. When this is applied properly the larvae are killed in a short time without injury to the plants. The method consists in placing a little carbon bisulphide in a small stender dish under the bell glass covering the culture. For a 5×9 inch bell glass about 1.5 cc. of the carbon bisulphide is sufficient. If larger doses are given, the plants are likely to be injured or killed.

The fluid soon evaporates, and after two or three hours the bell glass may be lifted for more complete ventilation. Carbon bisulphide is an effective and convenient fumigant because it vaporizes at low temperatures, and because the lethal dose for both pest and plant may easily be ascertained by trial.

A vigorously growing continuation shoot, if placed upon rich soil (preferably woods earth), may result in the growth shown in fig. 1. In form and size this figure shows the large liverwort-like branches with their massive midribs and ruffled margins. In many shoots, as was shown in a former publication, the branching is typically dichotomous. In the lower right hand corner of fig. 1 a portion has become detached from the parent system of shoots through death and decay of the oldest portion. It often happens that the plants so develop that the shoots become crowded and overlap (fig. 2). Owing to this overlapping some of the shoots become distorted, some growing upward into the air and developing numerous brown rhizoids from the upper as well as from the lower surface of the midrib. Archegonia are sometimes developed from both surfaces in such shoots, as well as upon those spread out flat, as in fig. 1.

Two other features of behavior appearing especially, although not exclusively, in the larger shoots are usually to be observed. They consist in the development of small proliferations springing from the surface of the midrib, from near the margins, or directly from the edges of the margins. Some of those from the margins are thin and irregular in outline, suggesting the tiny pinkish scales which develop from the margin of the thallus of *Marchantia*. These marginal scalelike proliferations are usually but one cell in thickness, and they are frequently white in color owing to lack of chloroplast development. Eventually they wither and dry up. They do not remain flat as they develop, but become rolled or folded.

The second kind of minute proliferation mentioned bears numerous antheridia. The antheridia-bearing proliferations arise from the midrib or from the wings of the thallus. It will be recalled that one of the important conditions necessary for continued growth of the prothallia or their clones is prevention of the development of embryo sporophytes. This can easily be accomplished if the plants are watered by sub-irrigation. In large and robust shoots no great

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(WITH TWO FIGURES)

Certain vigorous fern prothallia were transplanted to culture dishes upon rich soil and given plenty of space, and have been kept successfully in continuous growth for eight years. The plants themselves, or their continuation shoots (clones), have attained the size, and in general outline the appearance, of the liverwort *Marchantia* (fig. 1). Four years after the experiment was begun the results were published in this journal (**3**, **4**). The cultures have now been grown uninterruptedly for eight years, and thus the continuation shoots have been kept under constant observation. It would be possible to continue the cultivation indefinitely, if the difficulties encountered could be overcome.

In former publications the method of cultivation was described somewhat in detail, the difficulties which arose from time to time were mentioned, and remedies given. The most dangerous and annoying feature was the presence of the larvae of a small gnatlike dipterous insect (**4**). Fumigation of the greenhouse gave only temporary relief, for the gnats doubtless came from outside sources. They and their maggots appeared again in a comparatively short time, and it seemed that the cultures were doomed. Not only that, but new cultures started from spores were at times completely destroyed. In due time, however, and before the continuation cultures were seriously injured, an effective remedy was found which consisted in fumigating the cultures in which the larvae were found with the vapor of carbon bisulphide. When this is applied properly the larvae are killed in a short time without injury to the plants. The method consists in placing a little carbon bisulphide in a small stender dish under the bell glass covering the culture. For a 5×9 inch bell glass about 1.5 cc. of the carbon bisulphide is sufficient. If larger doses are given, the plants are likely to be injured or killed.

The fluid soon evaporates, and after two or three hours the bell glass may be lifted for more complete ventilation. Carbon bisulphide is an effective and convenient fumigant because it vaporizes at low temperatures, and because the lethal dose for both pest and plant may easily be ascertained by trial.

A vigorously growing continuation shoot, if placed upon rich soil (preferably woods earth), may result in the growth shown in fig. 1. In form and size this figure shows the large liverwort-like branches with their massive midribs and ruffled margins. In many shoots, as was shown in a former publication, the branching is typically dichotomous. In the lower right hand corner of fig. 1 a portion has become detached from the parent system of shoots through death and decay of the oldest portion. It often happens that the plants so develop that the shoots become crowded and overlap (fig. 2). Owing to this overlapping some of the shoots become distorted, some growing upward into the air and developing numerous brown rhizoids from the upper as well as from the lower surface of the midrib. Archegonia are sometimes developed from both surfaces in such shoots, as well as upon those spread out flat, as in fig. 1.

Two other features of behavior appearing especially, although not exclusively, in the larger shoots are usually to be observed. They consist in the development of small proliferations springing from the surface of the midrib, from near the margins, or directly from the edges of the margins. Some of those from the margins are thin and irregular in outline, suggesting the tiny pinkish scales which develop from the margin of the thallus of *Marchantia*. These marginal scalelike proliferations are usually but one cell in thickness, and they are frequently white in color owing to lack of chloroplast development. Eventually they wither and dry up. They do not remain flat as they develop, but become rolled or folded.

The second kind of minute proliferation mentioned bears numerous antheridia. The antheridia-bearing proliferations arise from the midrib or from the wings of the thallus. It will be recalled that one of the important conditions necessary for continued growth of the prothallia or their clones is prevention of the development of embryo sporophytes. This can easily be accomplished if the plants are watered by sub-irrigation. In large and robust shoots no great

FIGS. 1, 2.—Fig. 1 (left), clones of prothallia of *Matteuccia nodulosa* grown without appreciable crowding; fig. 2, culture of prothallial clones of same species which have become crowded in growth; slightly reduced.

care is necessary, since upon them only archegonia are produced. The entire tendency is toward femaleness. I have not found antheridia upon the midribs or the massive parts of such plants as are shown in fig. 1. On the contrary, when growth is less vigorous the small antheridia-bearing proliferations will appear. They may be numerous upon the upper side of older midribs which have begun to darken or to turn brown. To the unaided eye, or when viewed with the aid of a pocket lens, these antheridial outgrowths appear as yellowish-green granulations. They are literally covered with antheridia, the production of which exhausts these proliferations.

The most vigorous and rapidly growing specimens were, as a rule, purely female, antheridial outgrowths appearing upon those which were crowded or upon the older and doubtless more poorly nourished parts. These facts seem to lend strongest support to the theory that sex is purely quantitative and not qualitative. There is certainly no sex chromosome.

It is unnecessary to discuss the constantly increasing evidence concerning sex as a quantitative phenomenon in inheritance. The following comments will be confined to the prothallia of the species under consideration, *Matteuccia nodulosa* (*Onoclea struthiopteris*). For many years the writer has grown annually prothallia of this plant in many pure cultures. Careful observation of the spores shows that they vary in size, and, even when sown under the most favorable growth conditions, some of the spores give rise to plants of exceptional vigor, while other spores produce plants which are much less vigorous from the start. The larger and more robust plants develop into pure females, while the smaller ones may begin to bear antheridia when they are very minute, and continue to do so to the point of exhaustion, or to complete cessation of growth. It seems that in such plants the stimulus to develop antheridia far exceeds the stimulus leading to multiplication of vegetative cells. Of the large female specimens, that is, those bearing archegonia, only an occasional one produced a few antheridia upon the older parts. From a most critical study it was found that not more than 10 per cent of these large plants bore antheridia before the time of production of archegonia, and in many cases but one or two antheridia appeared. The cultures of the past eight years have shown that

large and vigorously growing plants developed only archegonia, and that when antheridial proliferations appeared they were found to develop from the older parts which were soon to die. When antheridia appeared upon the more thrifty plants, these sex organs were produced upon the weaker parts or upon the thinner marginal lobes. It seems reasonable to conclude that the more vigorous plants tend to remain female, and that production of antheridia is associated with less vigorous growth. Whether diminished vigor is the result of poorer nutrition in these plants, the writer does not know.

This study began eight years ago with the prothallia of two species, *Matteuccia nodulosa* and *Osmunda claytoniana*. During the past four years the cultures of *O. claytoniana* became weak, the majority dying; only a few clones now survive, and their growth is slow. In the earlier years these prothallia produced good vigorous cultures. Near the close of the fourth year, however, when prothallia were examined histologically, they were found to contain an endophytic fungus (4). Whether this endophyte contributed to the weakening of the plants the writer is unable to say. Endophytic fungi were not observed in prothallia of *Matteuccia*.

Summary

1. The continuation shoots (clones) of individual prothallia of *Matteuccia nodulosa* and *Osmunda claytoniana* have been grown in cultures for eight years (1922–1930).

2. Their continued existence was made possible by preventing the development of sporophytes. All conclusions reached at the end of the first four years of continuous cultivation were confirmed during the subsequent four years of observation.

3. The vigorous clones attained the size of the liverwort *Marchantia*, having a pronounced midrib and branching dichotomously.

4. When growth was most vigorous and rapid the clones bore, as a rule, only archegonia. Less favorable conditions of growth facilitated the production of antheridial proliferations. These developed mainly from the older parts of the midrib and from the thinner margins. The proliferations seem to be exhausted in the production of antheridia, as they did not continue to grow, but finally dried up.

5. This study lends unquestioned support to the theory that sex

is quantitative and not qualitative. The spores and their resulting gametophytes are bisexual haploids.

6. Vigorous growth results in the development of archegonia almost exclusively, while less vigorous growth leads to the production of antheridial proliferations also upon the same clones.

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CURRENT LITERATURE

BOOK REVIEW

International address list of botanists

One of the most important concrete results of the Fifth International Botanical Congress at Cambridge in 1930 was the decision to arrange for the publication of an international address list of botanists, to take the place of DÖRFLER'S *Botaniker Adressbuch*, which served the botanical world so well, but is now long out of date. The new address list will be on somewhat similar lines to DÖRFLER'S book and will contain the names of some 13,000–14,000 botanists and botanical institutions in all parts of the world. These will be arranged alphabetically by countries, and in most cases will be printed in the language of the country and will be provided with an index of personal entries and geographical indices. The price of the book is $3.25, the low cost being possible owing to the assistance received from the Bentham Trustees and the Carnegie Institution of New York. Advance orders are now being taken by Messrs. Bailliere, Tindall & Cox, 7 & 8 Henrietta Street, Covent Garden, London WC 2.